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(71) Applicant: THE GENERAL HOSPITAL CORPORATION [US/US]; 55 Fruit Street, Boston, MA 02114 (US).

(72) Inventor: NISHIMOTO, Ikuo; Lexington, MA 02173 (US).

(74) Agent: FRASER, Janis, K.; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).

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(54) Title: ASSAYS FOR G-PROTEIN-LINKED RECEPTORS

(57) Abstract

Chimeric polypeptides derived from the Gα subunits of various G-proteins, and methods of using such chimeric polypeptides in therapy and in screening potential therapeutic agents.

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ASSAYS FOR G-PROTEIN-LINKED RECEPTORS

The field of the invention is GTP-binding proteins and the receptors to which they link.

Background of the Invention

Of all the known membrane signal transducers, heteromeric GTP-binding proteins (G proteins) are the best characterized and the most versatile. They elicit biological functions which include hormone signalling, neurotransmission, chemotaxis, and perception of light, smell, and taste. G proteins couple to various cell surface receptors (G-linked receptors) and activate various intracellular effectors. Each G protein is made up of a Gα subunit and a Gβγ subunit. The specificity of G proteins' coupling to receptors and downstream signalling molecules is conferred by the various Gα subunits. The Gα molecules are classified into two categories: one is a class of sensory-organ-specific G proteins (e.g., $G\alpha_t$, $G\alpha_{olf}$, and $G\alpha_{gust}$), and the other is a less tissue-specific class consisting of $G\alpha_s$, the $G\alpha_i$ family (i.e., $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_{o1}$, $G\alpha_{o2}$, and $G\alpha_z$), the $G\alpha_{12}$ family (i.e., $G\alpha_{12}$ and $G\alpha_{13}$), and the $G\alpha_q$ family (i.e, $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$, and $G\alpha_{16}$). It is likely that more members of each class will be discovered.

Experiments using recombinant Gα chimeric molecules which have some peptide sequence derived from one type of Gα and additional sequence from another type of Gα (e.g., $G\alpha_{13}/\alpha_z$, $G\alpha_q/\alpha_{i2}$, and $G\alpha_{i2}/\alpha_{i1}$) have helped distinguish the receptor-specifying portions of these particular Gα molecules from their effector portions (Conklin et al., Nature 363: 274-276, 1993; Voyno-Yasenetskaya et al., J. Biol. Chem. 269: 4721-4724, 1994; Law et al., Mol. Pharmacol. 45: 587-590, 1994).

Furthermore, using a scanning mutagenesis approach, Berlot and Bourne (Cell 68: 911-922, 1992) have identified the shortest linear stretch (residues 236-356) in $G\alpha_s$ essential for $G\alpha_s$'s interaction with its effector, adenylyl cyclase.

Summary of the Invention

Applicants have established a $G\alpha_s$-based chimeric system for identifying the $G\alpha$ subunit of a G protein to which a given G-linked receptor couples. A series of $G\alpha_s/\alpha_x$ chimeras ($G\alpha_x$: any $G\alpha$ subunit except $G\alpha_s$) can be made with a first amino acid sequence corresponding to a region of $G\alpha_s$ (SEQ ID NO:21) encompassing $G\alpha_s$'s residues 236-356, followed by a second amino acid sequence 4-30 amino acids long and corresponding to a segment of $G\alpha_x$, which segment ends at (and includes) $G\alpha_x$'s C-terminal residue. The first amino acid sequence should contain the effector portion of $G\alpha_s$, and preferably will contain residues 1-389 of SEQ ID NO:21. The second amino acid sequence should contain the receptor-coupling portion of $G\alpha_x$, and preferably is 4 or 5 amino acids in length (e.g., as represented by SEQ ID NOs:22-30). Once the chimera is coupled to a $G\alpha_x$-coupled receptor via the $G\alpha_x$ portion of the chimera, the chimera can transduce a signal from the receptor to adenylyl cyclase (AC) via the $G\alpha_s$ portion of the chimera, resulting in an increase in cyclic AMP (cAMP) in the cell. Since the normal signalling pathway of non-chimeric $G\alpha_x$ does not involve AC, stimulation of the $G\alpha_x$-coupled receptor in the absence of the $G\alpha_s/\alpha_x$ chimera does not result in an increase in cellular cAMP.

In the present method, two identical samples of cells are provided, wherein the cells co-express a given G-linked receptor and a given $G\alpha_s/\alpha_x$ chimera. The second sample of cells is contacted with a ligand of the G-

linked receptor. AC activity, as manisfested by the rate of cAMP formation, is measured in both samples of cells. A significant increase in cAMP formation in the second sample as compared to the first sample indicates that that particular $G\alpha_x$ can couple to the receptor.

Cells co-expressing a given G-linked receptor and a given $G\alpha_s/\alpha_x$ chimera can be established by introducing into the cells a recombinant nucleic acid construct permitting expression of the receptor and a second recombinant nucleic acid construct permitting expression of the chimera. By "recombinant" is meant that the nucleic acid (or polypeptide) molecule is the product of artificial genetic manipulation.

As used herein, a $G\alpha_s/\alpha_x$ chimera is a polypeptide which includes the AC-coupling portion (e.g., amino acid residues 236-356) of $G\alpha_s$ (SEQ ID NO:21) as well as the receptor-coupling portion of $G\alpha_x$. The receptor-coupling portion can be 4-30 amino acids long and usually corresponds to the extreme C-terminal region of $G\alpha_x$. The chimeric polypeptide can also include an additional peptide sequence such as one that serves as an epitope tag, so long as the additional sequence does not interfere with the functioning of the chimera. By "G-linked receptor" is meant any naturally occurring cell surface receptor, or any functional recombinant variant thereof, that couples to a G protein. By "significant" is meant that the two values in comparison have a p value of less than 0.05 in Student's t test. By "ligand" is meant any molecule that binds and activates a receptor. A ligand can be, for example, the natural, physiological activator of the receptor (e.g., a hormone), a biologically active analogue thereof, or an antibody which binds to and thereby activates the receptor.

The chimeras of the invention can also be used in a method of screening compounds for their ability to

modulate the interaction between a given G-linked receptor and the Gα (i.e., $G\alpha_x$) subunit of a non-G_s G protein known to couple to the receptor. In the method, two identical samples of cells are provided, wherein the cells co-express the G-linked receptor and a $G\alpha_s/\alpha_x$ chimera. Both samples of cells are contacted with a ligand of the G-linked receptor. The second sample is additionally contacted with a test compound. cAMP formation is then measured in both samples. A significant decrease (or increase) of the cAMP level in the second sample as compared to the first sample indicates that the compound is capable of inhibiting (or enhancing) the interaction between the G-linked receptor and that particular $G\alpha_x$.

In this method, one can screen compounds that can modulate the following exemplary interactions: those between (1) $G\alpha_i$ and somatostatin receptor (SSTR) type 1, SSTR 3, insulin-like growth factor II receptor, muscarinic acetylcholine receptor, D_2-dopamine receptor, α_2-adrenergic receptor, adenosine receptor, thrombin receptor, or transforming growth factor β receptor; (2) $G\alpha_z$ and SSTR1; (3) SSTR3 and either $G\alpha_{14}$ or $G\alpha_{16}$; (4) SSTR5 and either $G\alpha_{12}$ or $G\alpha_{13}$; (5) $G\alpha_o$ and amyloid protein precursor (APP), transforming growth factor-β receptor, γ-butyric acid receptor, muscarinic acetylcholine receptor, adenosine receptor, thrombin receptor, or α_2-adrenergic receptor; or (6) $G\alpha_q$ and the T cell receptor, PTH/PTHrP receptor, calcitonin receptor, endothelin receptor, angiotensin receptor, platelet activating factor receptor, or thromboxane A_2 receptor. One can also use any constitutively active variants of these receptors, thereby eliminating the need for contacting the above-described cell samples with the receptors' ligands.

The chimeras have an inherent ability to alter the signal-transducing output of a given G-linked receptor. By "signal-transducing output" is meant the end result of the signalling initiated by a liganded G-linked receptor. Such an end result can be, for example, cell growth inhibition, cell proliferation, or secretion of a protein. To achieve this alteration, one can introduce into a target cell a Gα chimeric polypeptide containing the sequence of a Gα linking to a desirable effector, the receptor-coupling region (e.g., the 4-30 residues at the C-terminal end) of which sequence is replaced with that of a Gα to which the G-linked receptor normally couples. Such a chimeric polypeptide can be employed in a method of therapy for a condition associated with the function or lack of function of that receptor in a patient's cells. For instance, one can convert the activity of a constitutively active mutant of amyloid protein precursor (APP, known to couple to $G\alpha_o$) from AC-suppressing to AC-activating by supplying to a neural cell harboring the APP mutant a therapeutically effective amount of $G\alpha_s/\alpha_o$, e.g., by genetic therapy. The $G\alpha_s/\alpha_o$ polypeptide can be introduced into the target cell by introducing into the cell a recombinant nucleic acid construct that permits expression of the chimeric polypeptide. Such a construct can, for instance, be derived from a herpes simplex viral vector, or any other vector able to transfect neural cells.

Also within the invention is a method of improving the tumor growth inhibition ability of somatostatin (SST) or its known biologically active analogues. SST is known to inhibit growth of certain tumors, presumably by binding to SST receptors (SSTR) on the cell surface and inhibiting cell proliferation. It has been observed that in cancer treatments involving SST-related drugs, certain tumors become resistant to the drugs after a period of

time, presumably due to loss of SSTR5 expression on cell surface. Expression of a recombinant SSTR5 protein in the tumor cells can circumvent this problem. By "tumor growth inhibition" is meant that a tumor cell is prevented from proliferating or is induced to undergo apoptosis. Somatostatin is a 14 amino acid cyclic peptide hormone which was originally isolated from the hypothalamus. Biologically active analogues of SST include, but are not limited to, (1) naturally occurring analogues, such as SST-28 (FEBS Lett. 282: 363-367, 1991) and SST-25 (Gen CompEndocinol 81: 365-372, 1991); and (2) artificial compounds, such as octreotide (New Engl. J. Med. 334: 246-254, 1995), RC-160 (Buscail et al., PNAS 92: 1580-1584, 1995), RC-160-I and RC-160-II (Cancer Res. 54: 5895-5901, 1994), SMS 201-995 (Kubota et al., J. Clin. Invest. 93: 1321-1325, 1994), and BIM-23014 (i.e., lanreotide) (FASEB J. 7: 1055-1060, 1993).

Another method of inhibiting tumor growth is useful for tumor cells the growth of which is stimulated via an endogenous, hyperactive G-linked receptor. By "endogenous" is meant that the receptor is expressed in the cell absent any artificial genetic manipulation. By "hyperactive" is meant that the G-linked receptor is more active, or active for a longer period of time, than it is in a normal cell. Hyperactivity of a G-linked receptor can be caused by, for example, certain mutations in the receptor's peptide sequence, an unusually high level of the receptor's ligand, and/or a ligand that dissociates from the receptor at a rate lower than normal. One can then introduce into the tumor cell a $G\alpha_{12}$ or $G\alpha_{13}$ chimeric molecule, the C-terminal 5 residues of which are replaced with those of the $G\alpha$ that the hyperactive receptor normally couples to. Thus, the hyperactivity of the receptor is transduced via the $G\alpha_{12}$ or $G\alpha_{13}$ chimeric molecule to downstream growth-inhibitory effectors, which

counteract at least in part growth-stimulatory signals normally transduced by the receptor and its cognate G protein. Preferably, the chimera will transduce a signal that results in apoptosis of the tumor cell. The chimeric molecule can be introduced into the target cell *in vivo*, *in vitro*, or *ex vivo* in a carrier such as saline and/or liposomes. It can also be expressed by a recombinant nucleic acid construct that has been introduced into the cell.

Brief Description of the Drawings

Fig. 1 is a schematic representation of the $G\alpha_s$ chimeras constructed in the study. "$G\alpha_s$ wt" denotes wild-type $G\alpha_s$. Sequences of the last 5 C-terminal residues of the chimeras are illustrated, and referred to as SEQ ID NOs:22-31. These sequences are identical between $G\alpha_{i1}$ and $G\alpha_{i2}$, between $G\alpha_{o1}$ and $G\alpha_{o2}$, and between $G\alpha_q$ and $G\alpha_{11}$.

Fig. 2A is a bar graph showing the effects of SST on cholera toxin (CTX)-stimulated AC activity in cells expressing SSTR3. Cells were transfected with 0.125 μg of pCMV6-SSTR3 and 0.125 μg of pCMV6 vector. At 24 h after transfection, cells were treated for 30 min with or without 1 μM SST, in the presence of (1) 1 mM IBMX, or (2) 1mM IBMX plus 250 ng/ml CTX. cAMP formation was subsequently measured. All values are "means ± S.E." of quadruplicated experiments.

Fig. 2B & Fig. 2C are bar graphs showing the effects of SST on cAMP formation in cells expressing a $G\alpha_s$ chimera with (Fig. 2C) or without (Fig. 2B) SSTR3. Cells were transfected with 0.125 μg of plasmid encoding a $G\alpha_s$ chimera and 0.125 μg of either pCMV6-SSTR3 (Fig. 2C) or pCMV6 (Fig. 2B). At 24 h after transfection, cells were treated for 30 min with (1) 1 mM IBMX, or (2) 1 mM IBMX plus 1 μM SST. cAMP formation was subsequently

measured. AC activity levels are represented as percentage relative to the basal AC activity level in cells expressing $G\alpha_s/\alpha_{i1}$ alone. All values are "means ± S.E." of quadruplicated experiments. Similar results were found at least three times for each chimera.

Fig. 2D is a bar graph converted from Fig. 2B, showing the ratios of cAMP levels in the presence vs. absence of SST in transfected cells.

Fig. 3A & Fig. 3B are bar graphs showing the effects of SST on AC activity in cells expressing a $G\alpha_s$ chimera with (Fig. 3A) or without (Fig. 3B) SSTR3. Cells were transfected with 0.125 μg of plasmid encoding a $G\alpha$ chimera and either 0.125 μg of pCMV6-SSTR3 (Fig. 3A) or pCMV6 (Fig. 3B). Experiments were performed as described in the legend for Figs. 2A and 2B. AC activity levels are represented as percentage relative to the basal AC activity level in cells expressing $G\alpha_s/\alpha_q$ alone. All values are "means ± S.E." of quadruplicated experiments. Similar results were found at least three times for each chimera.

Fig. 3C is a bar graph showing the effects of SST on cAMP formation in cells expressing SSTR3 and a $G\alpha_s$ chimera derived from the $G\alpha_i$ or $G\alpha_q$ family. All the indicated chimeras were tested in parallel. Experiments were performed as described in the legend for Figs. 2A and 2B. All values are "means ± S.E." of quadruplicated experiments. Similar results were found at least three times for each chimera.

Fig. 3D is a bar graph converted from Fig. 3C, showing the ratios of cAMP levels in the presence vs. absence of SST in transfected cells.

Fig. 4A is a bar graph showing the stimulation of inositol phosphate (IP) production in cells transfected with (1) 0.125 μg of pCMV6-SSTR3, and (2) 0.125 μg of plasmid encoding the intact $G\alpha_{16}$. At 24 h after

transfection, cells were treated for 5 min with or without 1 μM SST, and IP production was measured. For treatment with pertussis toxin (PTX), at 24 h after transfection, cells were treated with 10 ng/ml PTX for 3 h and with SST as described above.

Fig. 4B is a bar graph showing the stimulation of IP production in cells transfected with (1) 0.125 μg of pCMV6-SSTR3, and (2) 0.125 μg of plasmid encoding intact $G\alpha_{14}$. Experiments were performed as described in Fig. 4A's legend.

Fig. 4C is a bar graph showing the stimulation of IP production in cells transfected with (1) 0.125 μg of pCMV6-SSTR3, and (2) 0.125 μg of plasmid encoding intact $G\alpha_q$. Experiments were performed as described in Fig. 4A's legend.

Fig. 4D is a bar graph showing the stimulation of IP production in cells transfected with (1) 0.125 μg of plasmid encoding parathyroid hormone receptor (PTHR), and (2) 0.125 μg of plasmid encoding intact $G\alpha_q$. Experiments were performed as described in Fig. 4A's legend.

Fig. 5A is a bar graph showing the effects of SST on AC activity in cells expressing a SSTR and $G\alpha_s/\alpha_{12}$. Cells were transfected with (1) 0.125 μg of plasmid encoding $G\alpha_s/\alpha_{12}$, and (2) 0.125 μg of pCMV6-SSTR1, pCMV6-SSTR2, pCMV6-SSTR3, pCMV6-SSTR5, pCDNAI-SSTR4, or pCMV6. At 24 h after transfection, cells were stimulated with 1 μM SST and cAMP formation was measured.

Fig. 5B is a bar graph converted from Fig. 5A, showing the ratios of cAMP levels in the presence vs. absence of SST.

Fig. 5C is a bar graph showing the effects of SST on AC activity in cells expressing a SSTR and $G\alpha_s/\alpha_{13}$. Cells were transfected with (1) 0.125 μg of plasmid encoding $G\alpha_s/\alpha_{13}$, and (2) 0.125 μg of pCMV6-SSTR1, pCMV6-

SSTR2, pCMV6-SSTR3, pCMV6-SSTR5, pCDNAI-SSTR4, or pCMV6. At 24 h after transfection, cells were stimulated with 1 μM SST and cAMP formation was measured.

Fig. 5D is a bar graph converted from Fig. 5C, showing the ratios of cAMP levels in the presence vs. absence of SST.

Detailed Description

Identification of the G Protein Gα Subunit that Associates with a Given G-Linked Receptor

One feature of the present invention is a comprehensive system wherein Gα subtype coupling can be assigned for any given G-linked receptor. The following examples are meant to illustrate, but not limit, the methods of the present invention. Other suitable modifications and adaptations of the conditions which are obvious to those skilled in the art are within the scope and spirit of the invention. For instance, genetically engineered variants of G-linked receptors can be substituted for the naturally occurring receptors. Standard transfection techniques other than the lipofection technique illustrated below, e.g., calcium phosphate precipitation, biolistic transfer, DEAE-Dextran, and viral-vector methods, can also be employed in the invention.

EXAMPLES

Materials and Methods

Cells and Transfection

COS cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and antibiotics, as described previously (Ikezu et al., J. Biol. Chem. 270: 29224-29228, 1995) Transient transfection was performed by lipofection as previously described (Ikezu et al., J. Biol. Chem. 270: 29224-29228,

1995). In brief, $2x10^4$ cells were seeded onto a 24-well plate and cultured in complete growth medium for 24 h. The cells were subsequently transfected with 0.25 μg of plasmid and 1 μl LipofectAMINE™ (GIBCO-BRL) for another 24 h in serum-free DMEM, and cultured in complete growth medium for an additional 24 h.

Measurement of AC activity

Intact-cell AC activity was assessed by measuring cAMP formation as described previously (Ikezu et al., J. Biol. Chem. 270: 29224-29228, 1995). In brief, at 24 h after transfection, cells were labeled with 6 μCi/ml of [^{3}H]adenine (Du Pont-NEN) for 24 h, and then treated with ligands of the G-linked receptor of interest (e.g., somatostatin-14 for a somatostatin receptor) and 1 mM IBMX (3-isobutyl-1-methylxanthine) for 30 min. The resultant radioactive cAMP was separated on two-step ion-exchange columns. Specific accumulation of cAMP was expressed as [cAMP/(ADP + ATP)] x 10^3, which represents intact-cell AC activity. Statistical analysis was performed with Student's t test.

Measurement of PI Turnover

PI (phosphatidyl inositol) turnover was assessed by measuring IP (inositol phosphates) production. $4x10^4$ cells were seeded onto a 24-well plate, cultured in complete growth medium for 24 h, and transfected for 24 h as described above. The culture medium was replaced with the labeling medium [inositol-free RPMI supplemented with dialyzed fetal calf serum and 10 μCi/ml of [^{3}H]myo-inositol (Du Pont-NEN)]. After incubation in the labeling medium at 37°C for 12 h, the cells were washed four times with inositol-free RPMI and treated with 1 μl somatostatin (SST) in inositol-free RPMI at 37°C for 5 min. After discarding the medium, the cells in 0.2 ml fresh medium were lysed on the plate by 0.8 ml of ice-cold 12.5% (final concentration: 10%) TCA, and the

lysate was put on ice for 20 min before centrifugation. Supernatant of the lysate was mixed well with 1 ml of saturated diethyl ether to extract acid. After 5 repeated extractions, the collected sample was neutralized with 1:100 dilution of concentrated ammonia, added to 4 ml water, and analyzed by a method described by Wu et al. (J. Biol. Chem. 267: 25798-25802, 1992) using Dowex column (AG 1-x8 Resin, 100-200 mesh, formate form, by BioRad).

Genes and Nucleic Acid Constructs

cDNA expression constructs encoding somatostatin receptors (SSTR) types 1, 2, 3, and 5 have been previously described (Kubota et al., J. Clin. Invest. 93: 1321-1325, 1994; Kagimoto et al., Biochem. Biophys. Res. Commun. 202: 1188-1195, 1994; Yamada et al., Mol. Endocrinol. 6: 2136-2142, 1992). These constructs (designated pCMV6-SSTR1, pCMV6-SSTR2, pCMV6-SSTR3, and pCMV6-SSTR5), all of which were derived from a pCMV6 vector (the SSTR1 and 2 constructs: pCMV6b; the SSTR3 and 5 constructs: pCMV6c), contain the SSTR coding sequences under the transcriptional control of the cytomegalovirus promoter. The SSTR4 expression construct (pcDNAI-SSTR4) was made by inserting the SSTR4 cDNA (Bito et al., J. Biol. Chem. 269: 12722-12730, 1994) in pBluescript (Strategene) into pcDNAI (Invitrogen).

The $G\alpha_s$ chimeras were constructed as follows. First, PCR was performed to add AflII and XbaI sites at the 3′ end of the wild type $G\alpha_s$ cDNA using the following two primers:

ATCTGGAATAACAGATGGCTGC (SEQ ID NO:1) and

AAACTAGTCTAGACTAGCTCAAATTCTTAAGTGCATGCGCTGGATGATGTCA (SEQ ID NO:2).

The PCR product was digested with BglII and XbaI, and subcloned into pcDNAI-$G\alpha_s$ (i.e., the original plasmid

containing the wild type Gαs cDNA) which had been predigested with the same enzymes. The resultant construct, designated $G\alpha_s$-AX, was sequenced to confirm the presence of AflII and XbaI sites. Subsequently, the construct was digested with AflII and XbaI, and ligated with two synthetic oligonucleotides to add sequence encoding the carboxyl-terminal five residues of a non-$G\alpha_s$ subunit. The oligonucleotides were:

(1) TTAAGAGATTGCGGCTTATTTTAAT (SEQ ID NO:3) and CTAGATTAAAATAAGCCGCAATCTC (SEQ ID NO:4) (for $G\alpha_s/\alpha_{i1}$);

(2) TTAAGAGAATGCGGCTTATTTTAAT (SEQ ID NO:5) and CTAGATTAAAATAAGCCGCATTCTC (SEQ ID NO:6) (for $G\alpha_s/\alpha_{i3}$);

(3) TTAAGAGGTTGCGGCTTGTACTAAT (SEQ ID NO:7) and CTAGATTAGTACAAGCCGCAACCTC (SEQ ID NO:8) (for $G\alpha_s/\alpha_o$);

(4) TTAAGATACATCGGTTTGTGTTAAT (SEQ ID NO:9) and CTAGATTAACACAAACCGATGTATC (SEQ ID NO:10) (for $G\alpha_s/\alpha_z$);

(5) TTAAGAGAGTACAACCTCGTTTAAT (SEQ ID NO:11) and CTAGATTAAACGAGGTTGTACTCTC (SEQ ID NO:12) (for $G\alpha_s/\alpha_q$);

(6) TTAAGAGATATCATGCTTCAATAAT (SEQ ID NO:13) and CTAGATTATTGAAGCATGATATCTC (SEQ ID NO:14) (for $G\alpha_s/\alpha_{12}$);

(7) TTAAGACAACTCATGCTTGAATAAT (SEQ ID NO:15) and CTAGATTATTCAAGCATGAGTTGTC (SEQ ID NO:16) (for $G\alpha_s/\alpha_{13}$);

(8) TTAAGAGAATTCAACTTAGTTTAAT (SEQ ID NO:17) and CTAGATTAAACTAAGTTGAATTCTC (SEQ ID NO:18) (for $G\alpha_s/\alpha_{14}$); and

(9) TTAAGAGAGATCAATTTGTTGTAAT (SEQ ID NO:19) and CTAGATTACAACAAATTGATCTCTC (SEQ ID NO:20) (for $G\alpha_s/\alpha_{16}$).

That the final products encoded the designed chimeric sequences was verified by sequencing. Creation of the AflII site in the $G\alpha_s$ cDNA did not change the encoded sequence, and thus did not affect the sequence of the $G\alpha_s/\alpha_x$ chimeras. Expression of the Gα chimeras was detected by a common Gα antibody (UBI) in immunoblot analysis. Rat parathyroid hormone (PTH) receptor cDNA was provided by Dr. G. V. Segre. Receptor ligands somatostatin-14 (SST-14, referred to as SST herein) and PTH 1-34 were purchased from Sigma. SST-28 was obtained from BACHEM.

Experimental Design and Results

Stimulation of $G\alpha_s$, but not any other Gα, results in an increase in adenylyl cyclase (AC) activity. All known types of AC can be stimulated by $G\alpha_s$. Thus, it is possible to monitor the activity of $G\alpha_s$ by measuring the rate of cAMP formation, a process catalyzed by AC.

It has been shown that the last 5 C-terminal residues of at least some of Gα's (e.g., $G\alpha_{i2}$ and $G\alpha_z$) is the major determinant for the subunit's receptor-coupling specificity (Conklin et al. Nature 363: 274-276, 1993 and references therein; Voyno-Yasenetskaya et al., J. Biol. Chem. 269: 4721-4724, 1994), and that a G-linked receptor has to recognize these C-terminal residues before it can exert its agonist-induced regulative effect. Thus, to assess the Gα-coupling ability of any non-AC stimulating (thus non-$G\alpha_s$-coupling) G-linked receptor, one can utilize $G\alpha_s/\alpha_x$ ($G\alpha_x$: any type of Gα except $G\alpha_s$) chimeras wherein the last five C-terminal residues of the $G\alpha_s$ polypeptide are replaced with those of $G\alpha_x$. If a receptor couples to $G\alpha_x$, it will, upon binding to its ligand, recognize and activate the $G\alpha_s/\alpha_x$ chimera, thereby resulting in $G\alpha_s$-mediated AC stimulation in the cell.

$G\alpha_s/\alpha_x$ chimeras consisting of $G\alpha_s$ 1-389 (which lacks the original five C-terminal residues of $G\alpha_s$) and the five C-terminal residues of each known $G\alpha$ were constructed. The five C-terminal residues are identical between $G\alpha_{i1}$ and $G\alpha_{i2}$, between $G\alpha_{o1}$ and $G\alpha_{o2}$, and between $G\alpha_q$ and $G\alpha_{11}$. Nine chimeras were constructed and designated $G\alpha_s/\alpha_{i1}$, $G\alpha_s/\alpha_{i3}$, $G\alpha_s/\alpha_o$, $G\alpha_s/\alpha_z$, $G\alpha_s/\alpha_q$, $G\alpha_s/\alpha_{12}$, $G\alpha_s/\alpha_{13}$, $G\alpha_s/\alpha_{14}$, and $G\alpha_s/\alpha_{16}$, respectively (Fig. 1). The residues 1-389 (SEQ ID NO:21) of $G\alpha_s$ are the following:

```
  1 MGCLGNSKTE DQRNEEKAQR EANKKIEKQL QKDKQVYRAT
 41 HRLLLLGAGE SGKSTIVKQM RILHVNGFNG EGGEEDPQAA
 81 RSNSDGEKAT KVQDIKNNLK EAIETIVAAM SNLVPPVELA
121 NPENQFRVDY ILSVMNVPDF DFPPEFYEHA KALWEDEGVR
161 ACYERSNEYQ LIDCAQYFLD KIDVIKQADY VPSDQDLLRC
201 RVLTSGIFET KFQVDKVNFH MFDVGGQRDQ RRKWIQCFND
241 VTAIIFVVAS SSYNMVIRED NQTNRLQEAL NLFKSIWNNR
281 WLRTISVILF LNKQDLLAEK VLAGKSKIED YFPEFARYTT
321 PEDATPEPGE DPRVTRAKYF IRDEFLRIST ASGDGRHYCY
361 PHFTCAVDTE NIRRVFNDCR DIIQRMHLR
```

The experimental strategy was to transiently express a $G\alpha_s/\alpha_x$ cDNA along with a SSTR cDNA, and then to compare AC activities in the presence and absence of SST. If treatment with SST promotes cAMP formation only in cells expressing the SSTR and a given $G\alpha_s/\alpha_x$, one can assume the linkage of the SSTR to that $G\alpha_s/\alpha_x$ and therefore to that $G\alpha_x$. The $G\alpha_s$ chimeras were each expressed as a 52-kDa protein at similar levels in COS cells, consistent with expected molecular weight.

The effect of SST on cAMP formation was first examined in cells transfected with a $G\alpha_s/\alpha_x$ construct alone (e.g., $G\alpha_s/\alpha_{i1}$, $G\alpha_s/\alpha_{i3}$, $G\alpha_s/\alpha_o$, $G\alpha_s/\alpha_z$, $G\alpha_s/\alpha_q$, $G\alpha_s/\alpha_{14}$, and $G\alpha_s/\alpha_{16}$). When an empty vector, rather than a SSTR cDNA construct, was transfected into these

chimera-expressing cells, SST had little effect on cAMP formation at up to
1 μM, as shown in Figs. 2B and 3B.

The next step was to confirm that the chimeras were functional. For this purpose, $G\alpha_s/\alpha_x$ chimeras where $G\alpha_x$ is derived from the $G\alpha_i$ family ($G\alpha_{i1}$, $G\alpha_o$, and $G\alpha_z$) were tested for their ability to transduce AC-stimulatory signal initiated by SST-bound SSTR3. SSTR3 has been shown to function as a G_i-coupled receptor and to suppress AC activity in various cell types (Yasuda et al., J. Biol. Chem. 267: 20422-20428, 1992; Yamada et al., Mol. Endocrinol. 6: 2136-2142, 1992; Kaupmann et al., FEBS Lett. 331: 53-59, 1993; Law et al., Mol. Pharmacol. 45: 587-590, 1993 and Law et al., Mol. Pharmacol. 45: 587-590, 1994; Patel et al., Biochem. Biophys. Res. Commun. 198: 605-612, 1994). Indeed, SST treatment resulted in inhibition of AC in COS cells transfected with a plasmid expressing SSTR3 (Fig. 2A). When cholera toxin, a potent stimulator of AC, was employed to increase the basal AC level, the inhibition of AC by SST treatment was even more apparent (Fig. 2A).

In clear contrast to the decrease in AC in cells expressing SSTR3 alone, SST augmented AC activity in cells co-expressing SSTR3 and either $G\alpha_s/\alpha_{i1}$ or $G\alpha_s/\alpha_{i3}$ (Fig. 2C). Thus, the interaction between SSTR3 and $G\alpha_s/\alpha_i$ chimeras converted the effect of SSTR3 activation from AC inhibition to AC stimulation by switching the effector region of the $G\alpha$ protein from that of $G\alpha_i$ to that of $G\alpha_s$, suggesting that the chimeras constructed herein were operative.

Notably, in cells expressing SSTR3 and either $G\alpha_s/\alpha_o$ or $G\alpha_s/\alpha_z$, no augmentation of AC was observed (Figs. 2C and 2D). These data were consistent with multiple reports demonstrating the linkage of SSTR3 solely to $G\alpha_{i1}$, $G\alpha_{i2}$, and $G\alpha_{i3}$ of the $G\alpha_i$ family, which is

known to have at least 6 members. Given the fact that the only Gα proteins known to inhibit AC are members of the $G\alpha_i$ family, which include the $G\alpha_i$'s (i.e., $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$), the $G\alpha_o$'s (i.e., $G\alpha_{o1}$ and $G\alpha_{o2}$), and $G\alpha_z$ (Wong et al., Nature 351: 63-65, 1991), the present data suggest that SSTR3 may inhibit cAMP formation exclusively through the $G\alpha_i$'s.

The next question was whether use of the remaining chimeras can reveal any unknown linkage of SSTR3 to other Gα's, particularly those in the $G\alpha_q$ family. For this purpose, an expression construct encoding SSTR3 and a second expression construct encoding one of $G\alpha_s/\alpha_q$, $G\alpha_s/\alpha_{14}$, and $G\alpha_s/\alpha_{16}$ were cotransfected into COS cells. cAMP formation in these cells was measured. In cells expressing SSTR3 and either $G\alpha_s/\alpha_{14}$ or $G\alpha_s/\alpha_{16}$, SST treatment resulted in small, but statistically significant increase in AC activity (Fig. 3A). Since SST significantly reduced cAMP formation when SSTR3 was transfected without $G\alpha_s/\alpha_{14}$ or $G\alpha_s/\alpha_{16}$ (Fig. 2A), it is conceivable that the net stimulation of AC by SSTR3 through these two chimeras may have been considerably larger than what was observed. In contrast, in cells co-expressing SSTR3 and $G\alpha_s/\alpha_q$, no stimulation of AC was observed under the same conditions.

Figs. 3C and 3D show results of the experiments wherein the linkage of SSTR3 to chimeras derived from the $G\alpha_i$ and $G\alpha_q$ families were examined in parallel. Again, the results demonstrated that SSTR3 may link to $G\alpha_{14}$ and $G\alpha_{16}$, in addition to the Gαi's, but not to any other members of the $G\alpha_i$ and $G\alpha_q$ families .

The putative linkage of SSTR3 to $G\alpha_{14}$ and $G\alpha_{16}$ was confirmed by use of the full length $G\alpha_{14}$ and $G\alpha_{16}$ molecules. It is known that, when linked to an appropriately liganded receptor, $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$ and $G\alpha_{16}$ are all able to stimulate phospholipase C (PLC). Thus, a

functional linkage between SSTR3 and any of these four molecules can be demonstrated by SST-initiated PI turnover in cells co-expressing SSTR3 and the Gα molecule. As shown in Fig. 4A, SST stimulated IP production when both $G\alpha_{16}$ and SSTR3 were expressed in COS cells. No PI turnover was observed when either molecule was expressed alone. In addition, consistent with the resistance of $G\alpha_{16}$ to pertussis toxin (PTX), PTX failed to affect this stimulation (Fig. 4A). Similarly, when SSTR3 was co-expressed with $G\alpha_{14}$, SST led to a statistically significant, though lower, increase of IP production (Fig. 4B). Again, no PI turnover was observed when either molecule was expressed alone. In contrast, when SSTR3 was co-expressed with $G\alpha_q$, SST had no effect on IP production (Fig. 4C). These data demonstrated that a linkage between a given G-linked receptor and a given $G\alpha_s/\alpha_x$ chimera is predictive of a linkage between the receptor and that particular $G\alpha_x$.

Under the same conditions, transfection of cDNA encoding the parathyroid hormone receptor (PTHR) with or without cDNA for $G\alpha_q$ resulted in significant stimulation of IP production in response to maximal PTH stimulation (Fig. 4D). Transfection of $G\alpha_q$ augmented the ability of PTHR to activate PLC. These data are consistent with the observations that (i) PTHR causes PI turnover in a PTX-insensitive manner (Iida-Klein et al., J. Biol. Chem. 270: 8458-8465, 1995), and (ii) COS cells endogenously express $G\alpha_q$ and $G\alpha_{11}$ (Wu et al., J. Biol. Chem. 267: 25798-25802, 1992). Therefore, SSTR3-mediated $G\alpha_{16}$ stimulation, which even exceeded PTHR-mediated $G\alpha_q$ stimulation, is specific and significant.

Interestingly, despite that SSTR3 coupled to $G\alpha_s/\alpha_{14}$ and $G\alpha_s/\alpha_{16}$ with similar efficiency (Figs. 3C and 3D), it coupled to intact $G\alpha_{16}$ far more efficient than to intact $G\alpha_{14}$ (Figs. 4A and 4B). This finding suggests that

the extreme C-terminal region of a Gα be the major but not sole determinant for the Gα's full interaction with its linked receptor. Other regions of the Gα polypeptide may also involve, as shown by a number of other studies.

In summary, inability of a G-linked receptor to couple to a given $G\alpha_s/\alpha_x$ indicates the inability of the receptor to recognize the C terminus of that particular $G\alpha_x$, and therefore rules out the coupling between the receptor and the intact $G\alpha_x$. In this context, the present study suggests for the first time that SSTR3 may not couple to $G\alpha_o$, $G\alpha_z$, $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{12}$, or $G\alpha_{13}$ (see below for $G\alpha_{12}$ and $G\alpha_{13}$). On the other hand, ability of a G-linked receptor to couple to a given $G\alpha_s/\alpha_x$ indicates that the receptor is capable of coupling to that particular $G\alpha_x$.

The present chimeric system is extremely useful in identifying potential receptor-Gα linkage, especially for Gα's which have less established signal-transducing effectors and which therefore are less amenable to assaying. In this regard, the present system can be employed to identify $G\alpha_{12}$- or $G\alpha_{13}$-coupled receptors. Although $G\alpha_{12}$ and $G\alpha_{13}$ have been implicated in pivotal cellular functions (Voyno-Yasenetskaya et al., Oncogene 9: 2559-2565, 1994 and Voyno-Yasenetskaya et al., J. Biol. Chem. 269: 4721-4724, 1994), receptors to which they couple remain elusive.

To investigate whether $G\alpha_{12}$ and $G\alpha_{13}$ couple to any of the 5 known subtypes of SSTR's, SST-induced cAMP formation was measured in cells co-expressing a given SSTR and either $G\alpha_s/G\alpha_{12}$ or $G\alpha_s/G\alpha_{13}$. Figs. 5A-5D shows that $G\alpha_s/G\alpha_{12}$ was activated by SSTR2, 4, and 5 in the order of SSTR5 >> SSTR2 ≈ SSTR4, while $G\alpha_s/G\alpha_{13}$ was activated almost exclusively by SSTR5. Notably, the stimulation of $G\alpha_s/G\alpha_{12}$ and $G\alpha_s/G\alpha_{13}$ by liganded SSTR5

yielded a more than 5 fold increase in the cAMP level (Figs. 5B and 5D).

Table I. Dose effects of SST (SST-14) and SST-28 on cAMP formation in chimera-expressing cells

Dose(M)	SSTR5 transfected cells		SSTR2 transfected cells	
	SST	SST-28	SST	SST-28
10^{-10}	104.1±4.5	98.9±2.1		
10^{-9}	90.4±4.3	95.2±4.1		
10^{-8}	102.5±4.2	98.5±8.6	94.1±9.0	107.0±3.3*
10^{-7}	282.3±2.1	359.8±3.6*	129.5±1.3	112.8±9.9*
10^{-6}	418.4±4.1	463.5±2.8*	202.5±15.3	187.7±2.9

After transfection of $G\alpha_s/\alpha_{12}$ chimera and SSTR2 or SSTR5 cDNA, cells were stimulated with various concentrations of SST-14 or SST-28, and cAMP formation was measured. The results are indicated as percentage relative to cAMP formation at 10^{-11} M SST or SST-28, which was similar to basal formation shown in Fig. 5. Data are presented as means ± S.E. of quadruplicated experiments.

Table I shows that, in the presence of $G\alpha_s/\alpha_{12}$, the stimulation of cAMP formation by SSTR5 and SSTR2 is SST-dosage-dependent and biphasic. At low SST concentrations, cAMP formation was slightly but reproducibly inhibited, whereas at higher concentrations, cAMP formation was strongly stimulated. It is conceivable that the former effect may be mediated by SSTR2/5's coupling to the endogenous $G\alpha_i$, and the latter

by SSTR2/5's coupling to $G\alpha_s/\alpha_{12}$. Several lines of evidence showed that the observed AC stimulation was not mediated by the $G\beta\gamma$ subunit released from the endogenous G_i.

Table I also indicates that SST-28 had a more potent effect on the function of SSTR5 than the naturally occurring SST (i.e., SST-14), regarding both of their inhibitory and stimulatory effects. These findings are consistent with the well known fact that SSTR5 has a higher affinity for SST-28 than for SST-14, while other SSTR's have a lower affinity for SST-28.

In addition to use in identifying a potential linkage between a given G-linked receptor and a given $G\alpha$ having less established signal-transducing effectors, the present system can also be used to investigate proteins with a G-linked-receptor-like structure (e.g., with multiple transmembrane domains) but having unknown functions. The system can also be used to investigate proteins which have only a single-transmembrane domain but which are suspected of being a G-linked receptor. Examples of these proteins are insulin-like growth factor II receptor (Murayama et al., J. Biol. Chem. 265: 17456-17462, 1990), amyloid precursor protein (APP) (Okamoto et al., FEBS Lett. 334: 143-148, 1995), and sperm β-1,4-galactosyltransferase (Gong et al., Science, 269: 1718-1721, 1995). There are many other single-spanning proteins with a possible G-coupling ability, examples of which include epidermal growth factor receptor (Sun et al., Proc. Natl. Acad. Sci. U.S.A., 92: 2229-2233, 1995), insulin receptor (Luttrell et al., J. Biol. Chem., 265: 16873-16879, 1990; Okamoto et al., FEBS Lett., 334: 143-148, 1994), and insulin-like growth factor I (IGF-I) receptor (Nishimoto et al., Biochem. Biophys. Res. Commun. 148: 407-412, 1987; Luttrell et al., J. Biol. Chem. 270: 16495-16498, 1995). The present system can be

employed to examine the Gα-coupling potential of these candidates as well.

Identification of Compounds Capable of Modulating the Interaction between a G-linked Receptor and Its Coupled Non-$G\alpha_s$ Gα Subunit

One aspect of the present invention is a method of identifying a compound that can modulate the interaction between a G-linked receptor and the Gα subunit of a non-G_s G protein known to couple to the receptor. In the claimed method, two samples of cells are provided, both of which express (a) the receptor of interest, and (b) a chimeric polypeptide containing amino acid residues 1-389 of $G\alpha_s$ (SEQ ID NO:21) followed by the C-terminal 5 amino acid residues of the non-$G\alpha_s$ Gα subunit known to couple to the receptor. A ligand of the G-linked receptor is administered to both cell samples. Prior to, subsequent to, or at the same time as the ligand administration, the second cell is contacted with a candidate compound. Then the activity of adenylyl cyclase in each cell sample is determined and compared as described above. A statistically significant (i.e., $p<0.05$ in Student's t test) change of the AC activity in the second cell sample as compared to the first cell sample indicates that the compound may be capable of modulating the interaction between the G-linked receptor and the coupling Gα subunit. For example, a statistically significant decrease of the AC activity in the compound-contacted cells will suggest that the compound may block the interaction. The efficacy of the compound can be confirmed by a second assay using the full length Gα subunit instead of the chimera.

Cell lines that can be used in connection with this method include those of mammalian origin, such as COS cells and HEK 293 cells (American Type Culture

Collection). Maintenance and transfection of such cells can be performed using well known methods. Proteins (a) and (b) (see above) can be introduced into the target cells via transfection of nucleic acid constructs encoding them. Techniques for making nucleic acid constructs are well known in the art (see, e.g., Sambrook et al., Molecular Cloning, a Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989); examples of such techniques have been illustrated above.

G-linked receptors of interest include, but are not limited to, those described in U.S. Patent No.5,559,209, herein incorporated by reference (e.g., insulin-like growth factor II receptor, muscarinic acetylcholine receptor, α_2-adrenergic receptor, adenosine receptor, thrombin receptor, transforming growth factor β receptor, T cell receptor, PTH/PTHrP receptor, calcitonin receptor, endothelin receptor, angiotensin receptor, platelet activating factor receptor, thromboxane A_2 receptor, any of the somatostatin receptors, D_2-dopamine receptor, γ-butyric acid receptor), and amyloid protein precursor (APP).

Nucleic acid constructs that permit expression of SSTR1, 3, and 5 in COS cells have been described above. APP has at least 10 isoforms, one of which (APP_{695}) is preferentially expressed in neuronal tissue (Sandbrink et al., J. Biol. Chem. 269: 1510, 1994). The construction of a baculovirus construct containing the APP_{695} cDNA has been described (Nishimoto et al., Nature 362: 75-79, 1993). Similar cloning techniques can be employed to create APP_{695} mammalian expression constructs based on mammalian expression vectors such as pCDNAI and pCMV6.

Constitutively active variants of the G-linked receptors can also be used in the present screening method, eliminating the need for their ligands. For

instance, three constitutively active APP_{695} mutants, designated Ile-APP, Phe-APP, and Gly-APP, have been identified in familial Alzheimer's Disease patients (Yamatsuji et al., Science 272: 1349-1352, 1996; and references therein). These three mutants have mis-sense mutations in which Val^{642} in the transmembrane domain of APP_{695} is replaced by Ile, Phe, or Gly, respectively.

Alteration of the Signal-Transducing Output of a G-Linked Receptor

The chimeras of the invention can alternatively be used in a method of altering the signal-transducing output of a G-linked receptor. Abnormalities of G-linked receptor functions have been implicated in many significant diseases such as familial Alzheimer's disease (Nishimoto et al., Nature 362: 75-79, 1993; Yamatsuji et al., Science 272: 1349-1352, 1996; Okamoto et al., The EMBO J. 15: 3769-3777, 1996; Ikezu et al., The EMBO J. 15: 2468-2475, 1996; and references therein), atherosclerosis, retinitis pigmentosa, malignant thyroid tumor, precocious puberty, and familial hypocalcineuric hypercalcemia (Clapham, Cell 75: 1237-1239, 1993; Lefkowitz, Nature 365: 603-604, 1993).

Amyloid protein precursor (APP), a G-linked cell surface receptor, has been shown to be mutated and constitutively active in at least some forms of familial Alzheimer's Disease (Okamoto et al., The EMBO J. 15: 3769-3777, 1996; and references therein). APP is known to couple to G_o, the activation of which inhibits adenylyl cyclase (Okamoto et al., The EMBO J. 15: 3769-3777, 1996 and references therein). Thus, changing the effector function of the G protein with which APP associates from inhibitory to stimulatory or neutral with regard to AC activity is expected to alleviate the symptoms of familial Alzheimer's Disease, and by

extension, any form of Alzheimer's Disease characterized by constitutive or other inappropriately activation of the receptor or its G protein.

The present invention provides a method for augmenting adenylyl cyclase activity in brain neurons of a mammal, and preferably, of a familial Alzheimer's patient. In this method, a $G\alpha_s$ subunit in which the C-terminal 5 aa residues are replaced with those of $G\alpha_o$ is introduced into the brain neurons of the mammal. This chimeric Gα molecule will compete with the endogenous $G\alpha_o$ for the binding of APP, and upon binding to APP, will transduce stimulatory signals to adenylyl cyclase, thereby counteracting the inhibitory signals transduced by native G_o. This chimeric molecule can be introduced into the target cell by overexpressing within the target cell a nucleic acid construct comprising a promoter sequence operably linked to a sequence encoding the protein. The nucleic acid construct is typically derived from a non-replicating linear or circular DNA or RNA vector, or from an autonomously replicating plasmid or viral vector; or the construct is integrated into the host genome. These nucleic acid constructs can be made with methods well known in the art (see, e.g., Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, New York, 1989). Any vector that can transfect a brain neuron may be used in the method of the invention. A preferred vector is a herpes simplex viral (HSV) vector or an appropriately modified version of this vector.

A therapeutic composition containing this vector may be used alone or in a mixture, or in chemical combination, with one or more materials, including other proteins or recombinant vectors that increase the biological stability of the recombinant vectors, or with materials that increase the therapeutic composition's

ability to penetrate the target tissue selectively. The therapeutic compositions of the invention is typically administered in a pharmaceutically acceptable carrier (e.g., physiological saline), which is selected on the basis of the mode and route of administration, and standard pharmaceutical practice. Suitable pharmaceutical carriers, as well as pharmaceutical necessities for use in pharmaceutical formulations, are described in *Remington's Pharmaceutical Sciences*, a standard reference text in this field, and in the USP/NF.

The therapeutic compositions of the invention can be administered in dosages determined to be appropriate by one skilled in the art. It is expected that the dosages will vary, depending upon the pharmacokinetic and pharmacodynamic characteristics of the particular agent, and its mode and route of administration, as well as the age, weight, and health of the recipient; the nature and extent of the disease; the frequency and duration of the treatment; the type of, if any, concurrent therapy; and the desired effect.

The therapeutic compositions may be administered to a patient by any appropriate mode, e.g., via applying drops or spray onto the nasal mucosa, or via injection into the nasal mucosa, as determined by one skilled in the art. Alternatively, it may be desired to administer the treatment surgically to the target tissue. The treatments of the invention may be repeated as needed, as determined by one skilled in the art.

Inhibition of Tumor Growth

By using the chimeric Gα system of the present invention, $G\alpha_{12}$ and $G\alpha_{13}$ have been shown to couple to SSTR5 (see above). These two Gα's, which have been implicated as transducing apoptosis-generating and cell-proliferation-inhibiting signals, are ubiquitously

expressed in human cells. Thus, the invention includes a method of inhibiting tumor growth by expressing an exogenously introduced SSTR5 protein, e.g., a recombinant protein comprising (a) SSTR5, or (b) a biologically active fragment thereof, in a tumor cell. Recombinant $G\alpha_{12}$ or $G\alpha_{13}$ polypeptides can also be introduced into the target cell. Upon administration of SST or its biologically active analogue, the recombinant SSTR5 present on the cell surface will be stimulated and will thereby inhibit growth of the tumor cell via endogenous or recombinant $G\alpha_{12}$ and $G\alpha_{13}$.

This aspect of the invention is useful in cancer treatments using SST-related drugs (i.e., SST or SST analogues). Such treatments frequently lead to loss of SSTR's naturally expressed on cancer cells, thereby desensitizing the cells to the SST-related drugs. Introduction of recombinant SSTR5 into the cancer cells solves this problem, at least temporarily; further transfetions may be necessary to maintain the effect, if the recombinant SSTR5 is lost as well. All cancers, including highly malignant ones such as pancreatic cancer and small cell lung cancer, can be treated by the present method. The recombinant SSTR5 protein can be introduced into the cancer cells by overexpressing within the cells a nucleic acid construct comprising a mammalian promoter sequence operably linked to a sequence encoding the protein. Preferably, the construct primarily targets fast-proliferating cells, and can, for example, be derived from retroviral, adenoviral, adeno-associated-viral, or herpes simplex viral vectors, or any appropriately modified versions of these vectors. Retroviral vectors are particularly appropriate, as they selectively integrate into the genome of replicating cells, such as tumor cells. Methods for constructing expression vectors are well known in the art (see, e.g.,

Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989). The administration of SST, or its analogue, and a therapeutic composition comprising the SSTR5 construct can be conducted using guidelines described in the previous section.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: The General Hospital Corporation

(ii) TITLE OF THE INVENTION: G-LINKED RECEPTORS

(iii) NUMBER OF SEQUENCES: 31

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Fish & Richardson P.C.
(B) STREET: 225 Franklin Street
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02110-2804

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/028,340
(B) FILING DATE: 11-OCT-1996

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Fraser, Janis K
(B) REGISTRATION NUMBER: 34,819
(C) REFERENCE/DOCKET NUMBER: 08472/706WO1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617-542-5070
(B) TELEFAX: 617-542-8906
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATCTGGAATA ACAGATGGCT GC 22

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AAACTAGTCT AGACTAGCTC AAATTCTTAA GTGCATGCGC TGGATGATGT CA 52

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTAAGAGATT GCGGCTTATT TTAAT 25

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTAGATTAAA ATAAGCCGCA ATCTC 25

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTAAGAGAAT GCGGCTTATT TTAAT 25

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTAGATTAAA ATAAGCCGCA TTCTC 25

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TTAAGAGGTT GCGGCTTGTA CTAAT 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CTAGATTAGT ACAAGCCGCA ACCTC 25

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTAAGATACA TCGGTTTGTG TTAAT 25

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CTAGATTAAC ACAAACCGAT GTATC 25

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TTAAGAGAGT ACAACCTCGT TTAAT 25

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CTAGATTAAA CGAGGTTGTA CTCTC 25

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTAAGAGATA TCATGCTTCA ATAAT 25

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTAGATTATT GAAGCATGAT ATCTC 25

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TTAAGACAAC TCATGCTTGA ATAAT 25

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTAGATTATT CAAGCATGAG TTGTC 25

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TTAAGAGAAT TCAACTTAGT TTAAT 25

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTAGATTAAA CTAAGTTGAA TTCTC 25

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTAAGAGAGA TCAATTTGTT GTAAT 25

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CTAGATTACA ACAAATTGAT CTCTC 25

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 389 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Met Gly Cys Leu Gly Asn Ser Lys Thr Glu Asp Gln Arg Asn Glu Glu
 1               5                  10                  15
Lys Ala Gln Arg Glu Ala Asn Lys Lys Ile Glu Lys Gln Leu Gln Lys
            20                  25                  30
Asp Lys Gln Val Tyr Arg Ala Thr His Arg Leu Leu Leu Leu Gly Ala
        35                  40                  45
Gly Glu Ser Gly Lys Ser Thr Ile Val Lys Gln Met Arg Ile Leu His
    50                  55                  60
Val Asn Gly Phe Asn Gly Glu Gly Gly Glu Glu Asp Pro Gln Ala Ala
65                  70                  75                  80
Arg Ser Asn Ser Asp Gly Glu Lys Ala Thr Lys Val Gln Asp Ile Lys
                85                  90                  95
Asn Asn Leu Lys Glu Ala Ile Glu Thr Ile Val Ala Ala Met Ser Asn
            100                 105                 110
Leu Val Pro Pro Val Glu Leu Ala Asn Pro Glu Asn Gln Phe Arg Val
        115                 120                 125
Asp Tyr Ile Leu Ser Val Met Asn Val Pro Asp Phe Asp Phe Pro Pro
    130                 135                 140
Glu Phe Tyr Glu His Ala Lys Ala Leu Trp Glu Asp Glu Gly Val Arg
145                 150                 155                 160
Ala Cys Tyr Glu Arg Ser Asn Glu Tyr Gln Leu Ile Asp Cys Ala Gln
                165                 170                 175
Tyr Phe Leu Asp Lys Ile Asp Val Ile Lys Gln Ala Asp Tyr Val Pro
            180                 185                 190
Ser Asp Gln Asp Leu Leu Arg Cys Arg Val Leu Thr Ser Gly Ile Phe
        195                 200                 205
```

```
Glu Thr Lys Phe Gln Val Asp Lys Val Asn Phe His Met Phe Asp Val
    210                 215                 220
Gly Gly Gln Arg Asp Gln Arg Arg Lys Trp Ile Gln Cys Phe Asn Asp
225                 230                 235                 240
Val Thr Ala Ile Ile Phe Val Val Ala Ser Ser Ser Tyr Asn Met Val
                245                 250                 255
Ile Arg Glu Asp Asn Gln Thr Asn Arg Leu Gln Glu Ala Leu Asn Leu
            260                 265                 270
Phe Lys Ser Ile Trp Asn Asn Arg Trp Leu Arg Thr Ile Ser Val Ile
        275                 280                 285
Leu Phe Leu Asn Lys Gln Asp Leu Leu Ala Glu Lys Val Leu Ala Gly
    290                 295                 300
Lys Ser Lys Ile Glu Asp Tyr Phe Pro Glu Phe Ala Arg Tyr Thr Thr
305                 310                 315                 320
Pro Glu Asp Ala Thr Pro Glu Pro Gly Glu Asp Pro Arg Val Thr Arg
                325                 330                 335
Ala Lys Tyr Phe Ile Arg Asp Glu Phe Leu Arg Ile Ser Thr Ala Ser
            340                 345                 350
Gly Asp Gly Arg His Tyr Cys Tyr Pro His Phe Thr Cys Ala Val Asp
        355                 360                 365
Thr Glu Asn Ile Arg Arg Val Phe Asn Asp Cys Arg Asp Ile Ile Gln
    370                 375                 380
Arg Met His Leu Arg
385
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Asp Cys Gly Leu Phe
 1               5
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Glu Cys Gly Leu Tyr
 1               5
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Gly Cys Gly Leu Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Tyr Ile Gly Leu Cys
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Glu Tyr Asn Leu Val
1 5

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Asp Ile Met Leu Gln
1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Gln Leu Met Leu Glu
1 5

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Glu Phe Asn Leu Val
1 5

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Glu Ile Asn Leu Leu
1 5

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: N/A
(D) TOPOLOGY: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Gln Tyr Glu Leu Leu
1 5

We claim:

1. A method of determining whether a given G-linked receptor associates with the Gα subunit of a non-G_s G protein, said method comprising:

(1) providing a first cell and a second cell of the same cell type, each of which expresses

(a) said receptor, and

(b) a chimeric polypeptide comprising

(i) a first amino acid sequence corresponding to residues 236-356 of SEQ ID NO:21, and

(ii) a second amino acid sequence 4-30 amino acids in length and corresponding to a segment of said Gα subunit, which segment ends at and includes the C-terminal residue of said Gα subunit;

(2) contacting said second cell with a ligand of said receptor; and

(3) comparing the activity levels of adenylyl cyclase in said first and second cells, wherein a higher level in said second cell than in said first cell indicates that said receptor associates with said Gα subunit.

2. The method of claim 1, wherein said first amino acid sequence comprises amino acid residues 1-389 of SEQ ID NO:21, and said second amino acid sequence comprises the C-terminal 5 amino acid residues of said Gα subunit.

3. The method of claim 2, wherein said C-terminal 5 amino acid residues are selected from the group consisting of SEQ ID NOs: 22-30.

4. A method of identifying a compound that can modulate the interaction between a given G-linked receptor and the Gα subunit of a non-G_s G protein known to couple to said receptor, said method comprising:

(1) providing a first cell and a second cell of the same cell type, each of which expresses

(a) said receptor, and

(b) a chimeric polypeptide comprising

(i) a first amino acid sequence corresponding to residues 236-356 of SEQ ID NO:21, and

(ii) a second amino acid sequence 4-30 amino acids in length and corresponding to a segment of said Gα subunit, which segment ends at and includes the C-terminal residue of said Gα subunit;

(2) contacting said first cell with a ligand of said receptor;

(3) contacting said second cell with said ligand in the presence of a candidate compound; and

(4) comparing the activity levels of adenylyl cyclase in said first and second cells, wherein a higher or lower level in said second cell than in said first cell indicates that the compound modulates said interaction.

5. The method of claim 4, wherein said first amino acid sequence comprises amino acid residues 1-389 of SEQ ID NO:21, and said second amino acid sequence comprises the C-terminal 5 amino acid residues of said Gα subunit.

6. The method of claim 4, wherein said receptor is somatostatin receptor type 1 and said Gα subunit is $G\alpha_i$ or $G\alpha_z$.

7. The method of claim 4, wherein said receptor is somatostatin receptor type 3 and said Gα subunit is $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_{14}$, or $G\alpha_{16}$.

8. The method of claim 4, wherein said receptor is somatostatin receptor type 5 and said Gα subunit is $G\alpha_{12}$ or $G\alpha_{13}$.

9. The method of claim 4, wherein said receptor is insulin-like growth factor II receptor, muscarinic acetylcholine receptor, D_2-dopamine receptor, α_2-adrenergic receptor, adenosine receptor, thrombin receptor, or transforming growth factor β receptor; and said Gα subunit is $G\alpha_{i1}$, $G\alpha_{i2}$, or $G\alpha_{i3}$.

10. The method of claim 4, wherein said receptor is amyloid protein precursor (APP), transforming growth factor-β receptor, γ-butyric acid receptor, muscarinic acetylcholine receptor, adenosine receptor, thrombin receptor, or α_2-adrenergic receptor; and said Gα subunit is $G\alpha_o$.

11. The method of claim 4, wherein said receptor is the T cell receptor, PTH/PTHrP receptor, calcitonin receptor, endothelin receptor, angiotensin receptor, platelet activating factor receptor, or thromboxane A_2 receptor; and said Gα subunit is $G\alpha_q$.

12. A method of identifying a compound that can modulate the interaction between $G\alpha_o$ and a constitutively active mutant of APP, said method comprising:

(1) providing a first cell and a second cell of the same cell type, each of which expresses

(a) said mutant, and

(b) a chimeric polypeptide comprising

(i) a first amino acid sequence corresponding to residues 236-356 of SEQ ID NO:21, and

(ii) a second amino acid sequence 4-30 amino acids in length and corresponding to a segment of $G\alpha_o$, which segment ends at and includes the C-terminal residue of said $G\alpha_o$;

(2) contacting said second cell with a candidate compound; and

(3) comparing the activity levels of adenylyl cyclase in said first and second cells, wherein a higher or lower level in said second cell than in said first cell indicates that the compound modulates said interaction.

13. The method of claim 12, wherein said first amino acid sequence comprises amino acid residues 1-389 of SEQ ID NO:21, and said second amino acid sequence comprises the C-terminal 5 amino acid residues of $G\alpha_o$.

14. The method of claim 12, wherein said mutant is Ile-APP, Phe-APP, or Gly-APP.

15. A method of altering the signal-transducing output of a given G-linked receptor in a cell, said method comprising introducing into the cell a chimeric polypeptide comprising:

(a) a first polypeptide having the contiguous sequence of a 4 or 5 residue C-terminal segment of a first Gα subunit, wherein said first Gα subunit is a Gα subunit to which said receptor naturally links; and

(b) a second polypeptide having the entire, except for 4 or 5 C-terminal residues, contiguous sequence of a second Gα subunit, wherein said second Gα subunit, when activated, leads to a signal-transducing output different from that of said first Gα subunit;

provided that (1) if said first Gα subunit is $G\alpha_i$, said second Gα subunit cannot be $G\alpha_q$; and (2) if said first Gα subunit is $G\alpha_{13}$, said second Gα subunit cannot be $G\alpha_z$.

16. The method of claim 15, wherein said receptor is APP, Ile-APP, Phe-APP, or Gly-APP; said first Gα subunit is $G\alpha_o$; and said second Gα subunit is $G\alpha_s$.

17. The method of claim 16, wherein the cell is a neural cell of a mammal.

18. The method of claim 17, wherein said mammal is an Alzheimer's Disease patient.

19. A nucleic acid molecule comprising a promoter operably linked to a sequence encoding a chimeric polypeptide comprising (a) amino acid residues 1-389 of SEQ ID NO:21, and (b) an amino acid sequence representing the C-terminal 5 residues of a naturally occurring Gα polypeptide that is not $G\alpha_s$.

20. The nucleic acid molecule of claim 19, wherein said C-terminal 5 residues are selected from the group consisting of SEQ ID NOs:22-30.

21. A method of inhibiting the growth of a tumor cell, said method comprising:

(1) introducing into the tumor cell (a) a somatostatin receptor type 5 polypeptide, or (b) a nucleic acid molecule that directs the expression of said polypeptide in the cell; and

(2) contacting the cell with somatostatin or a biologically active analogue of somatostatin.

22. The method of claim 21, wherein said tumor cell is a human small cell lung cancer cell.

23. The method of claim 22, wherein said nucleic acid molecule comprises a viral vector.

24. The method of claim 21, said method comprising the additional step of, prior to said contacting step, introducing into the tumor cell (1) a $G\alpha_{12}$ or $G\alpha_{13}$ polypeptide, or (2) a nucleic acid molecule that directs the expression of said $G\alpha_{12}$ or $G\alpha_{13}$ polypeptide in the cell.

25. A method of inhibiting the growth of a tumor cell, the growth of which is stimulated via an endogenous, hyperactive G-linked receptor, said method comprising introducing into said tumor cell a polypeptide comprising (1) an amino acid sequence representing the C-terminal 4 or 5 contiguous residues of a $G\alpha$ that naturally couples to said G-linked receptor; and

(2) an amino acid sequence representing the entire, except the C-terminal 4 or 5 residues, contiguous sequence of $G\alpha_{12}$ or $G\alpha_{13}$.

26. A nucleic acid molecule comprising a promoter operably linked to a sequence encoding a chimeric polypeptide comprising:

(1) an amino acid sequence representing the C-terminal 4 or 5 residues of a first $G\alpha$ subunit;

(2) an amino acid sequence representing all except the C-terminal 4 or 5 residues of a second $G\alpha$ subunit, said second $G\alpha$ subunit being $G\alpha_{12}$ or $G\alpha_{13}$;

provided that when said first $G\alpha$ subunit is $G\alpha_z$, said second $G\alpha$ subunit cannot be $G\alpha_{13}$.

27. A polypeptide the amino acid sequence of which comprises:

(1) a sequence representing the C-terminal 4 or 5 contiguous residues of a first Gα subunit that naturally couples to said G-linked receptor;

(2) a sequence representing all except the C-terminal 4 or 5 residues of a second Gα subunit, said second Gα subunit being $G\alpha_{12}$ or $G\alpha_{13}$;

provided that when said first Gα subunit is $G\alpha_z$, said second Gα subunit cannot be $G\alpha_{13}$.

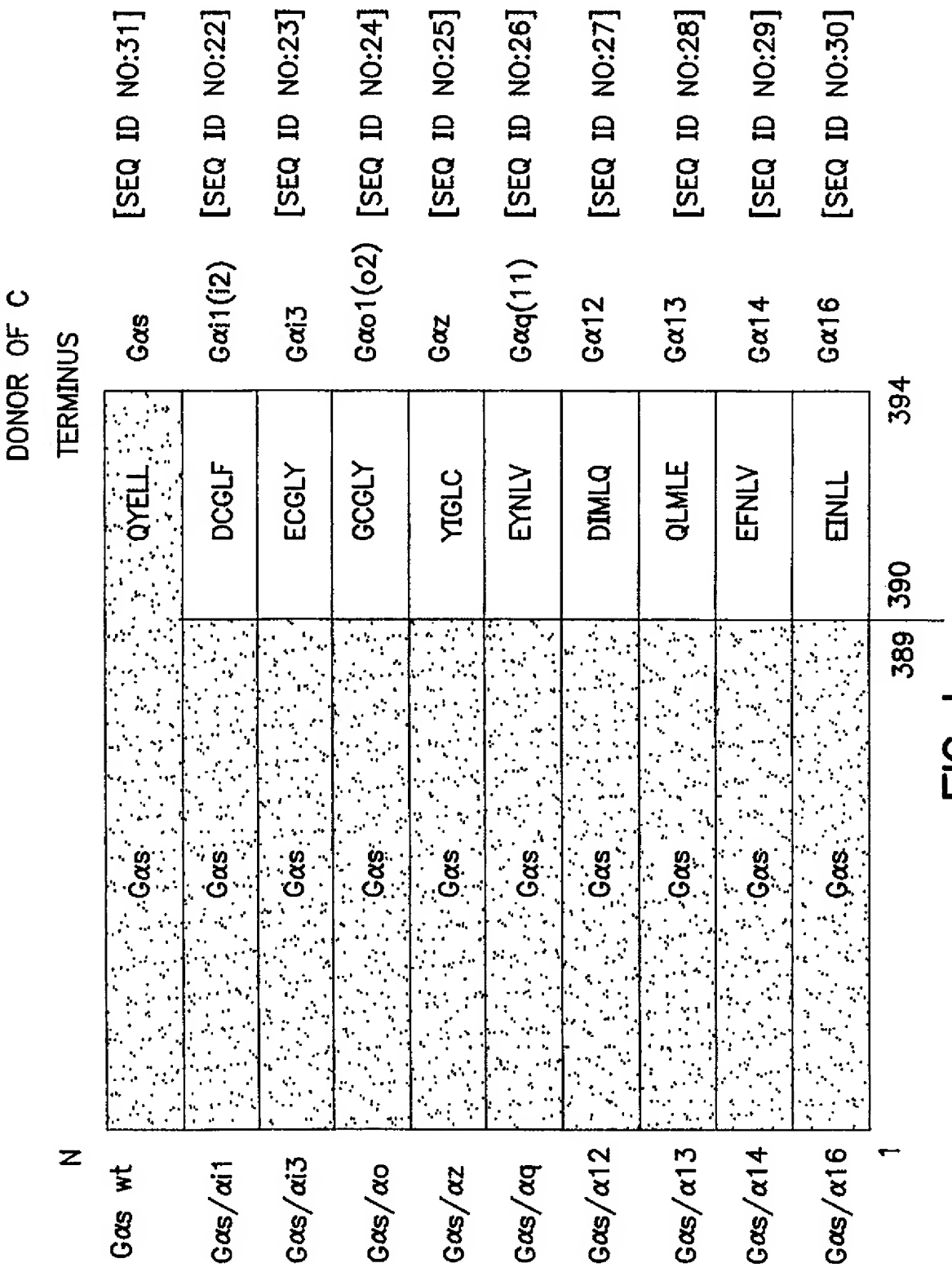


FIG. 1

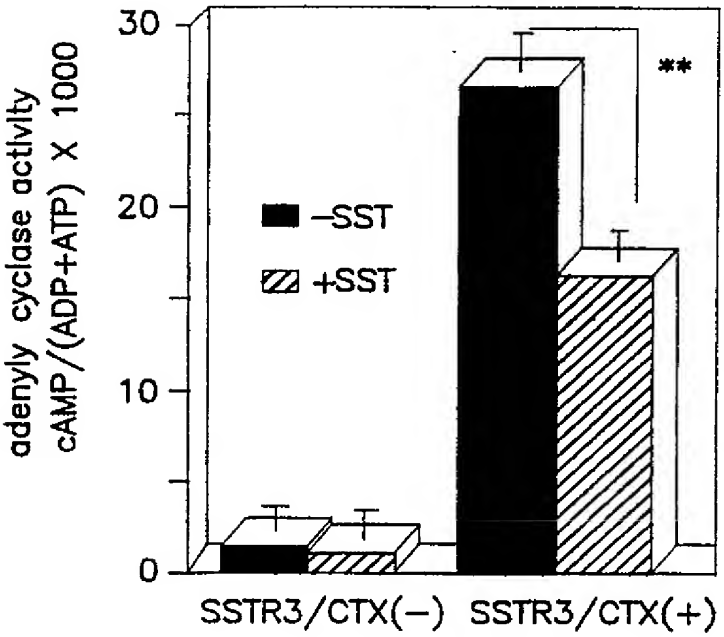


FIG. 2A

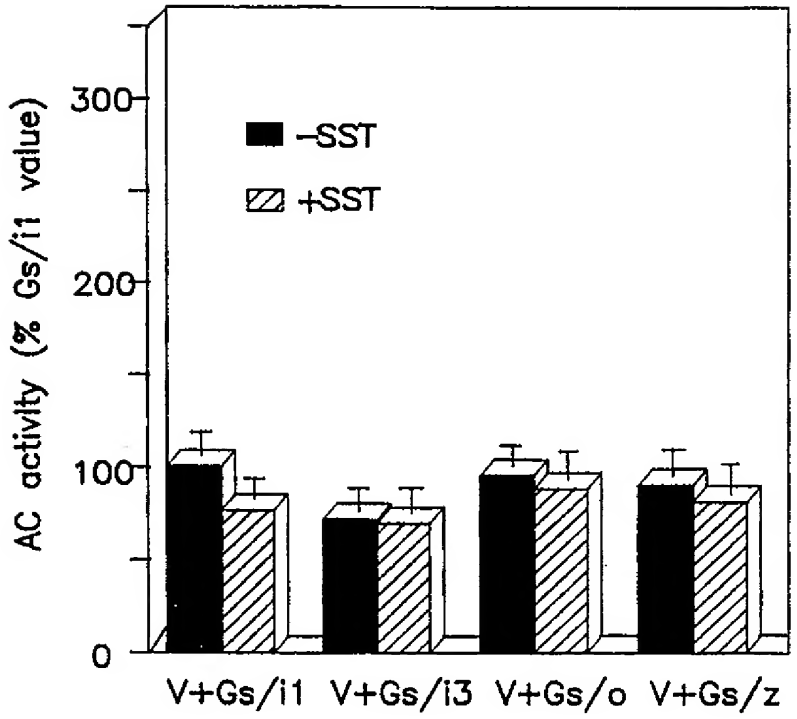
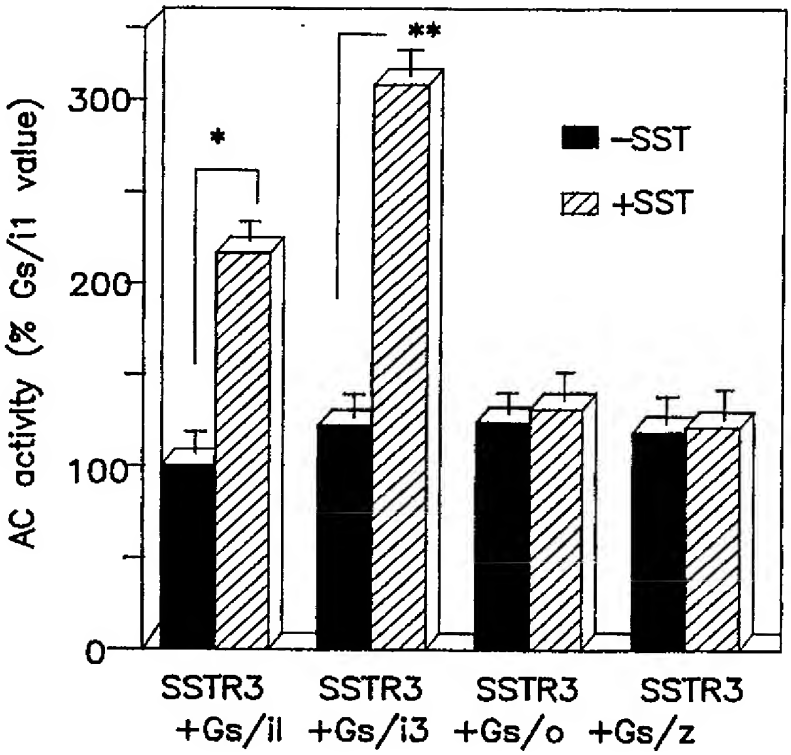


FIG. 2B

FIG. 2C



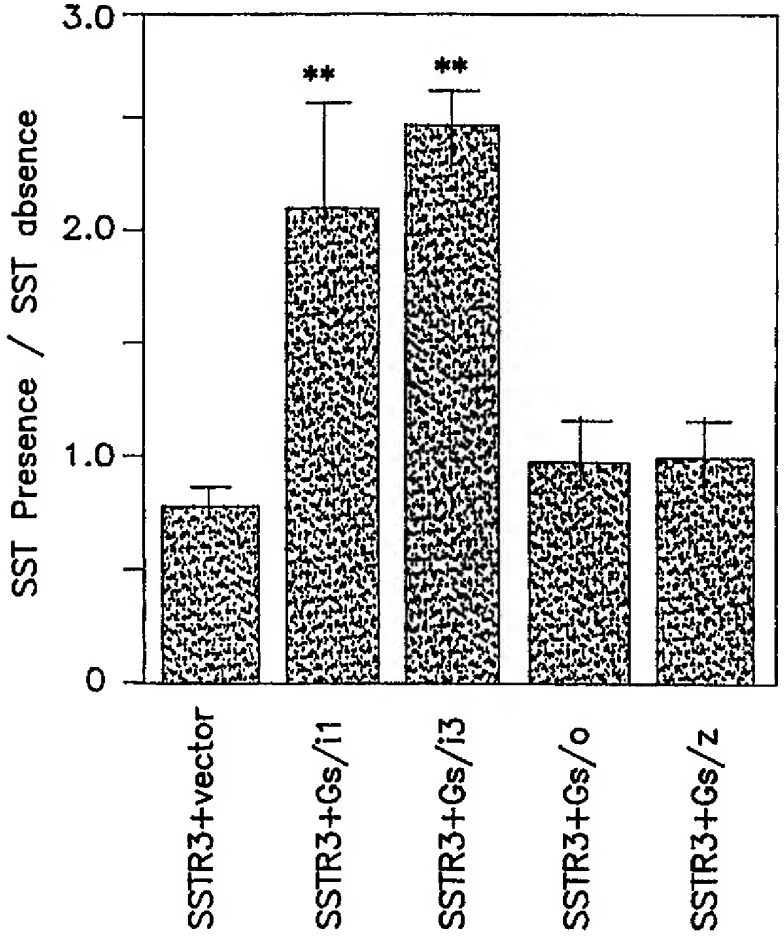


FIG. 2D

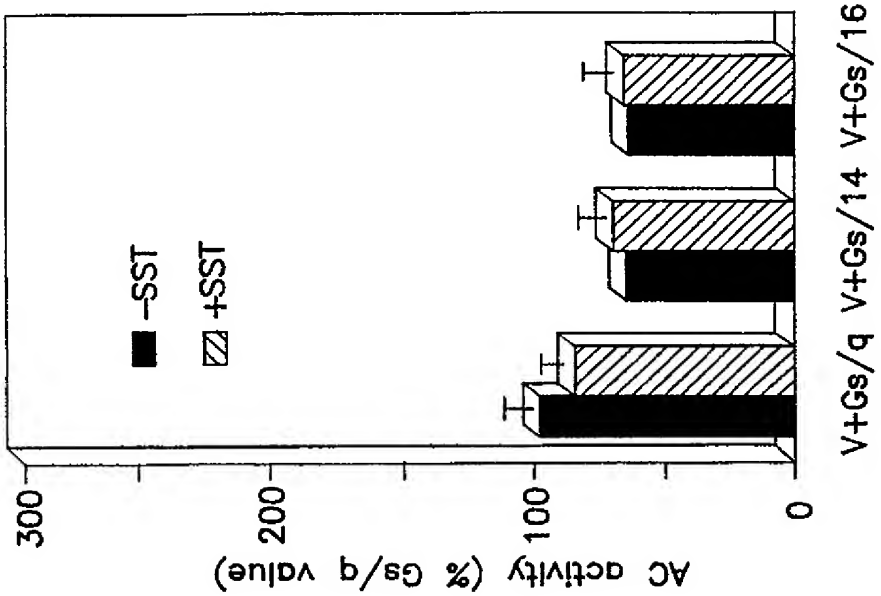

AC activity (% Gs/q value)
300
200
100
0
-SST
+SST
V+Gs/q
V+Gs/14
V+Gs/16

FIG. 3B

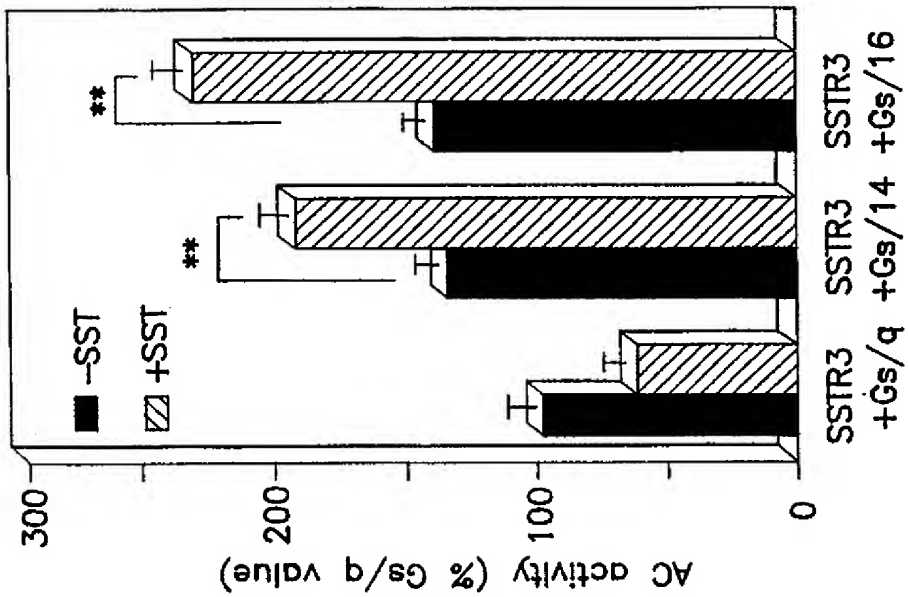

AC activity (% Gs/q value)
300
200
100
0
-SST
+SST
**
**
SSTR3 +Gs/q
SSTR3 +Gs/14
SSTR3 +Gs/16

FIG. 3A

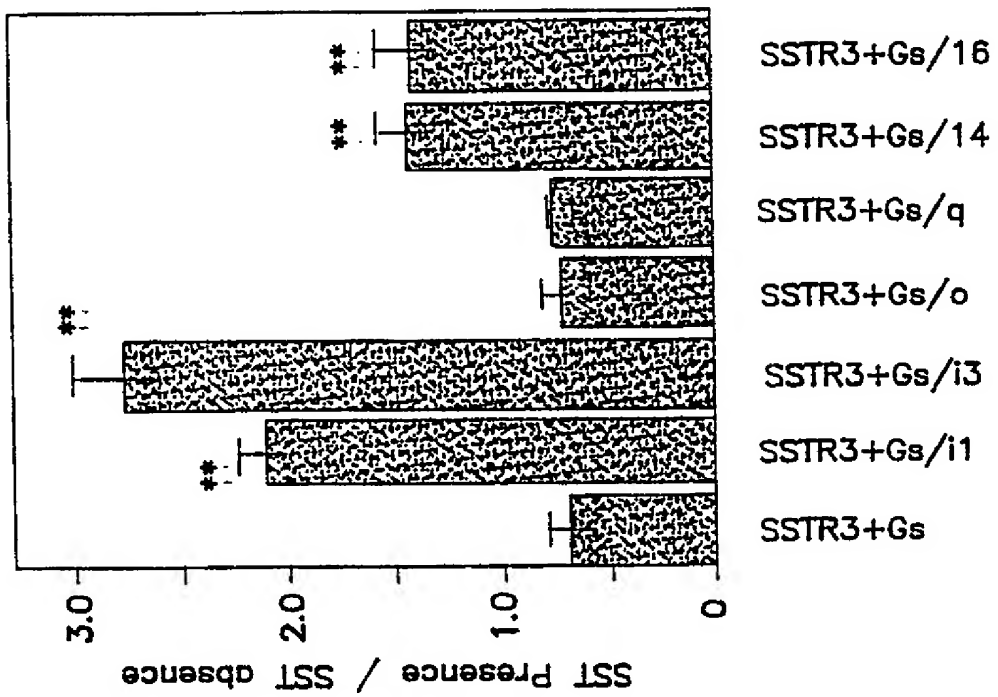


FIG. 3D

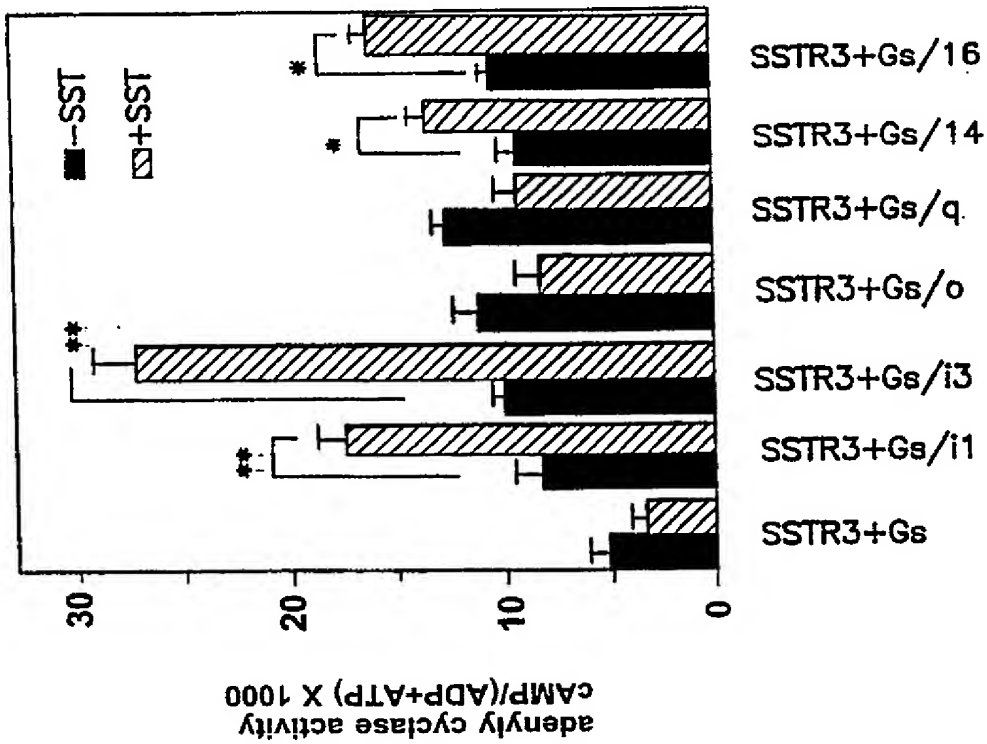


FIG. 3C

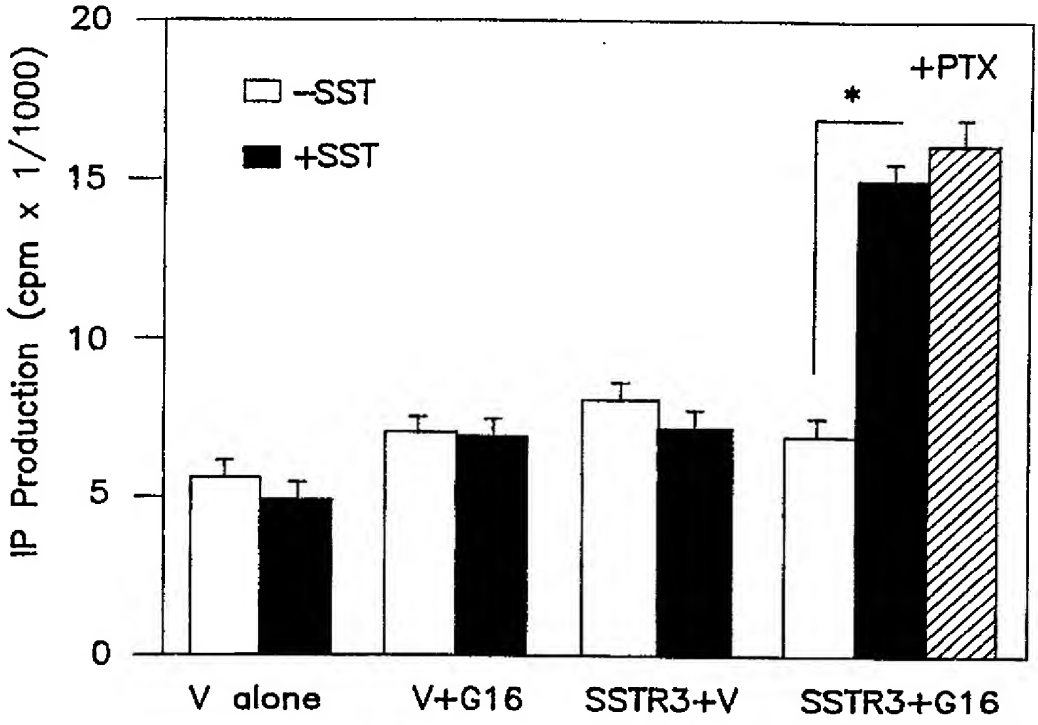

20
15
10
5
0
IP Production (cpm x 1/1000)
-SST
+SST
+PTX
*
V alone
V+G16
SSTR3+V
SSTR3+G16

FIG. 4A

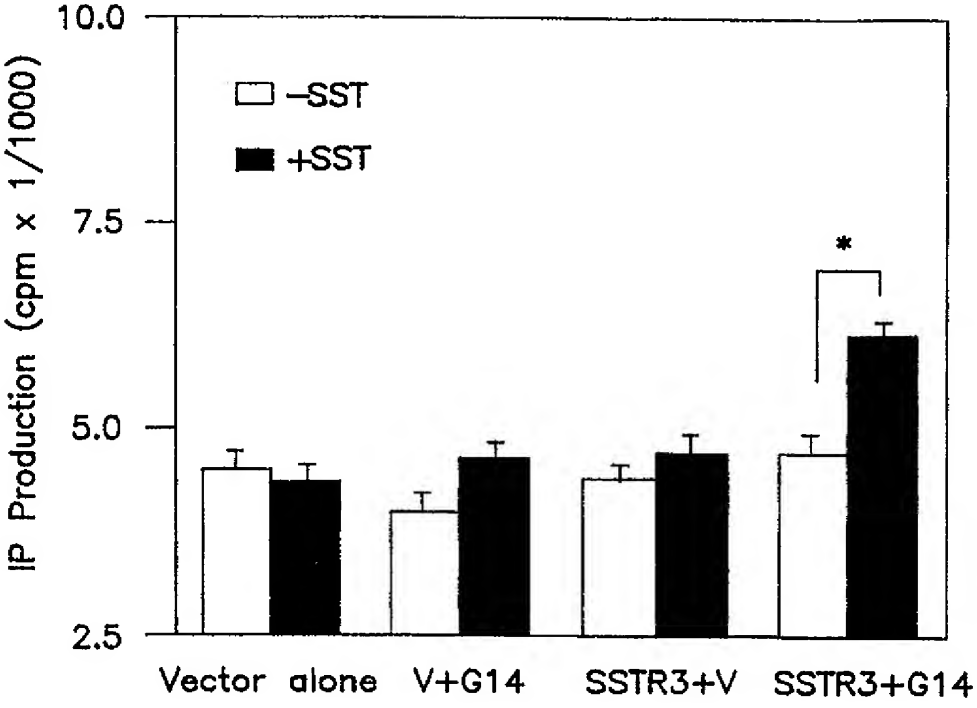

10.0
7.5
5.0
2.5
IP Production (cpm x 1/1000)
-SST
+SST
*
Vector alone
V+G14
SSTR3+V
SSTR3+G14

FIG. 4B

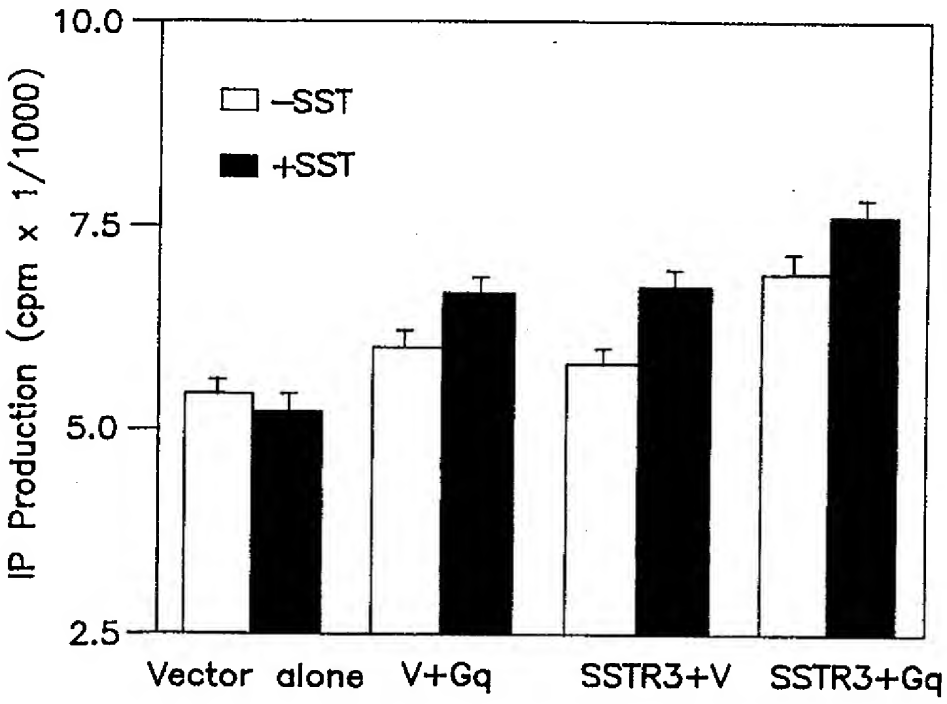


FIG. 4C

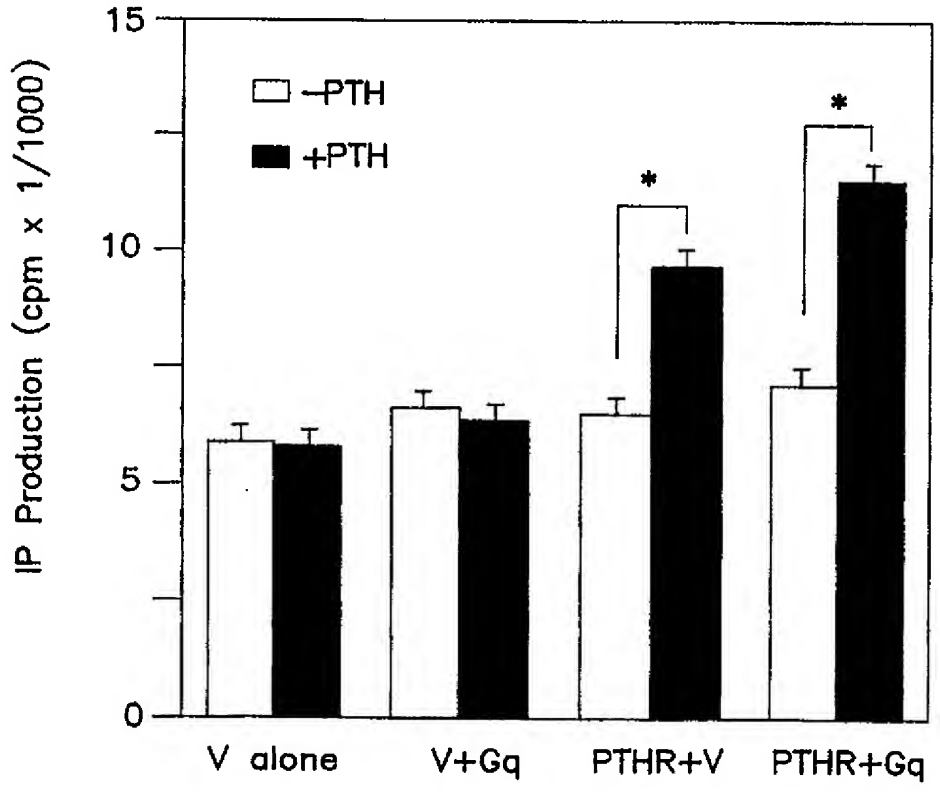


FIG. 4D

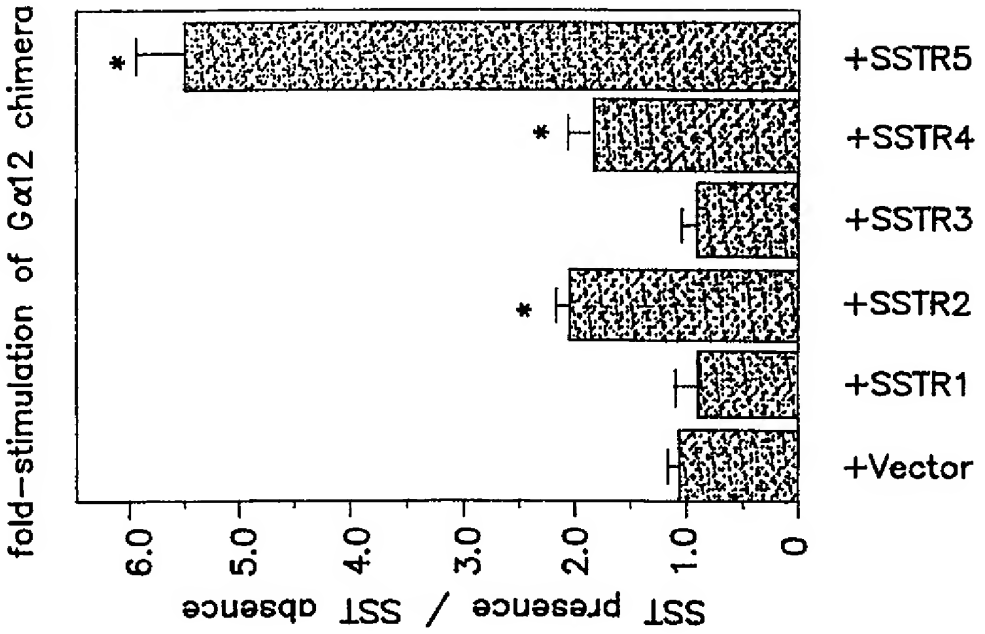

fold-stimulation of Gα12 chimera
SST presence / SST absence
6.0
5.0
4.0
3.0
2.0
1.0
0
+SSTR5
+SSTR4
+SSTR3
+SSTR2
+SSTR1
+Vector

FIG. 5B

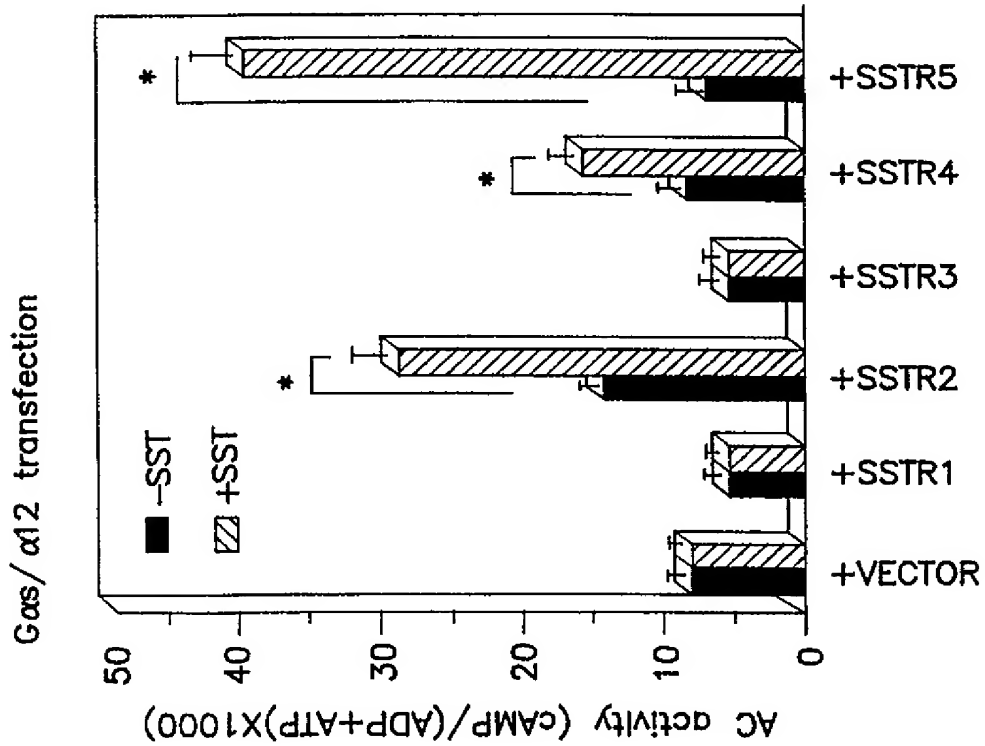

Gαs/α12 transfection
AC activity (cAMP/(ADP+ATP)X1000)
50
40
30
20
10
0
-SST
+SST
+SSTR5
+SSTR4
+SSTR3
+SSTR2
+SSTR1
+VECTOR

FIG. 5A

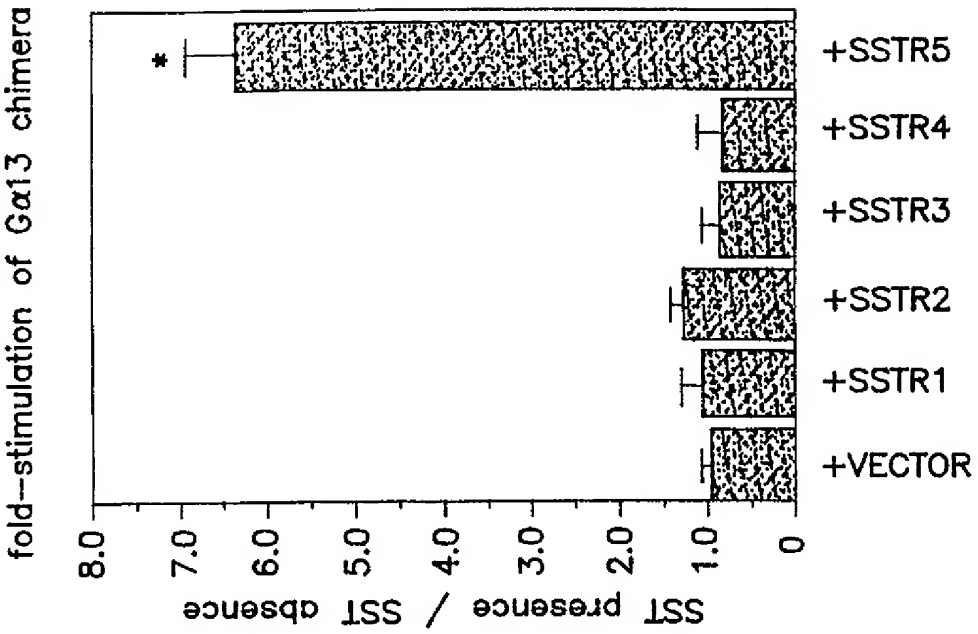


FIG. 5D

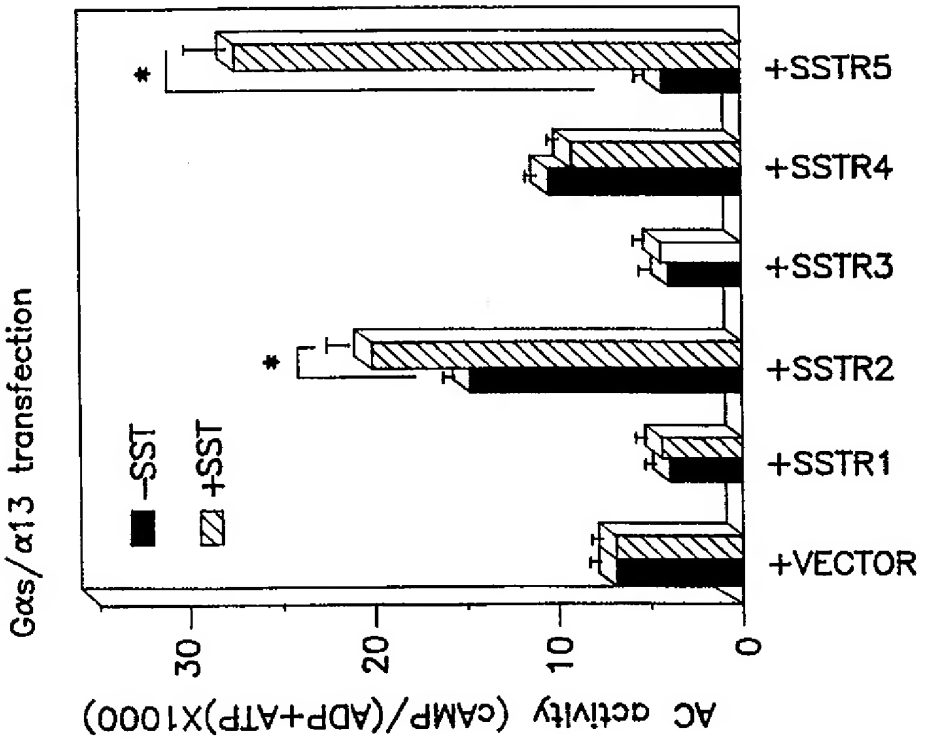


FIG. 5C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/20510

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 14/705; C12N 15/12, 15/62; C12Q 1/02; G01N 33/53
US CL :435/ 7.1, 29, 69.7; 530/350; 536/23.4.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/ 7.1, 29, 69.7; 530/350; 536/23.4.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y ----- A	BERLOT, C.H. et al. Identification of effector-activating residues of $G_{s\alpha}$. Cell. 06 March 1992, Vol. 68, pages 911-922, especially page 911, 912, 919, and 920.	1-7, 9-20, 26, 27 ----- 8
Y ----- A	CONKLIN, B.R. et al. Substitution of three amino acids switches receptor specificity of $G_q\alpha$ to that of $G_i\alpha$. Nature. 20 May 1993, Vol. 363, pages 274-276, especially page 274, figure 1.	1-7, 9-20, 26, 27 ----- 8
Y ----- A	VOYNO-YASENETSKAYA, T. et al. Gα13 stimulates Na-H exchange. J. Biol. Chem. 18 February 1994, Vol. 269, No. 7, pages 4721-4724, especially page 4723, figure 3.	26, 27 ----- 1-20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 27 FEBRUARY 1997	Date of mailing of the international search report 01 APR 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MICHAEL D. PAK Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/20510

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y ----- A	LAW, S.F. et al. $G_{i\alpha 1}$ selectively couples somatostatin receptor subtype 3 to adenylyl cyclase: identification of the functional domains of this α subunit necessary for mediating the inhibition by somatostatin of cAMP formation. Molecular Pharmacology. April 1994, Vol. 45, No. 4, pages 587-590, especially page 589, figures 2 and 3.	1-7, 9-20, 26, 27 ----- 8
Y ------ A	US 5,559,209 A (NISHIMOTO) 24 September 1996 (24.09.96), especially columns 1-3.	1-7, 9-18 ----- 8
Y, P	US 5,578,451 A (NISHIMOTO) 26 November 1996 (26.11.96), especially columns 1-4.	10, 12-18
Y	YAMATSUJI, T. et al. G protein-mediated neuronal DNA fragmentation induced by familial Alzheimer's disease-associated mutants of APP. Science. 31 May 1996, Vol. 272, pages 1349-1352, especially pages 1349 and 1351.	10, 12-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/20510

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-20, 26, 27

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/20510

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN-BIOSCIENCE, CAPLUS, MEDLINE, BIOSIS, EMBASE, SCISEARCH, SWISS-PROT, A-GENESEQ, PIR.

search terms: g-protein, chimer?, c-termin?

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-19, 20, 26, and 27, drawn to a method of determining whether a given G-linked receptor associates with the G-alpha subunit, a method of identifying a compound that can modulate the interaction between a given G-linked receptor associates with the G-alpha subunit, a method of identifying a compound that can modulate the interaction between a G-alpha-o and a constitutively active mutant of APP, a method of altering the signal-transducing output of a given G-linked receptor in a cell, a nucleic acid molecule, and polypeptide..

Group II, claims 21-24, drawn to a method of inhibiting the growth of a tumor cell with somatostatin.

Group III, claim 25, drawn to a second method of inhibiting the growth of a tumor cell.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons.

The special technical feature of Group I is a method using the specific G-protein, a nucleic acid, and polypeptide. Pursuant 37 CFR 1.475(d), these claims are considered by the ISA/US to constitute the main invention, and none of the related groups II-III correspond to the main invention.

The product of Group I does not share the same special technical feature as the methods of groups II and III because the product of group I is not used in or produced by the methods of groups II and III, and each defines a separate invention over the art.

The methods of Groups I, II, and III, do not share a special technical feature in any pairing because the methods have materially different process steps and are practiced for materially different purposes, and each defines a separate invention over the art.

Since no special technical feature of any group other than the main invention is shared by any of the other inventions, unity of invention is lacking.